The American Journal of Medicine

VOL. XXIII AUGUST, 1957 NO. 2

Editorial

Anions Versus Cations?

A COMMENDABLE trend, discussed here a few years ago [*1*], urges us to stop calling anions *acids* and cations *bases*. Perhaps it is in this spirit that several writers now refer to acid-base balance as *anion-cation* balance. The result, however, is just opposite to the one presumably intended: more clearly than ever anions are equated with acidity, cations with basicity, and acid-base balance as a war between the opposed anions and cations.

The present-day student [*2*] finds the unitarian approach to hydrogen-ion control far more satisfactory. Communication must, however, be preserved between the two viewpoints; our students still need to understand how it is possible to discuss the subject in terms of fixed ions, and some of their seniors should perhaps note that the subject can, if necessary, be discussed without reference to fixed ions! With this need in mind, I would like to add to my earlier discussion [*3*] this hypothetic dialogue:

ANTAGONIST: If the concept of anion-cation balance is defective, why does it work out as well as it does?

REPLY: Because fixed anions and cations are carefully defined so that in conjunction their concentrations reflect the level of the real actors, namely the buffer anions and through them the hydrogen ion. Under these conditions one gets the impression that the fixed ions are producing or combating disturbances of the acid-base balance.

ANTAGONIST: But do not serum analyses show metabolic acidosis to be caused either by a high chloride level (that is, a hyperchloremic acidosis) or by some other fixed anion?

REPLY: On the contrary I would view the elevated anion concentrations as a *result*, not a *cause* of the acidosis. An acid HX, is the cause. The H^+ which it provides is the real hazard. At the moment of analysis this H^+ has mainly disappeared by reacting with buffer anions, whereas X^- is still present. Therefore, we tend to blame X^-.

ANTAGONIST: Consider then that 0.9 per cent NaCl is an acidifying body fluid because of its high chloride content. To permit it to become neutral, we need to create a gap between the Na^+ and Cl^- concentrations, for example by removing chloride. Such a gap automatically will be filled with bicarbonate ion in the biologic environment. Does not this show that chloride is acidifying?

REPLY: True, at the high CO_2 pressure of our internal environment, "physiological" saline solution becomes quite acid. Such a solution cannot form a single bicarbonate ion without also forming a H^+. It is these hydrogen ions which must be extracted from it before it can be neutral. The removal of HCl (but not merely of Cl^-) would accomplish this purpose. But note that this limitation does not arise from the chloride ion level.

ANTAGONIST: Consider also that the ultimate correction of metabolic acidosis is renal, not respiratory, because only the kidneys can excrete the acidifying fixed anions.

REPLY: The respiratory correction is non-ultimate because it cannot actually excrete hydrogen ions; it merely renders most of them

[1] RELMAN, A. S. What are acids and bases? *Am. J. Med.*, 17: 435, 1954.

[2] KAUFMAN, H. E. and ROSEN, S. W. Clinical acid-base regulation—the Bronsted schema. *Surg., Gynec. & Obst.*, 103: 101, 1956.

[3] CHRISTENSEN, H. N. Control of the hydrogen ion. *New England J. Med.*, 247: 174–175, 1952.

innocuous by the reactions, $H^+ + HCO_3^- \rightleftharpoons H_2CO_3 \rightleftharpoons CO_2\uparrow + H_2O$. These two reactions must, however, be reversed to restore the normal bicarbonate level; hence the hydrogen ion ultimately reappears. It is for this reason that the kidney must excrete all the H^+ of the invading acid.

ANTAGONIST: But is it not by excreting the excess anion that the kidney corrects the acidosis? As the urine is acidified, the doubly-charged phosphate ion is converted to the singly-charged form, so that more fixed anion can be excreted or, what amounts to the same thing, more fixed cation conserved.

REPLY: Instead I prefer to say that as the H^+ concentration is increased in the renal tubular lumen by secretory activity, $HPO_4^=$ is converted to $H_2PO_4^-$. In this way more H^+ is excreted to help correct the acidosis. The adjustment in Cl^- and B^+ excretion seems to me incidental.

ANTAGONIST: How then would you explain the place of ammonia synthesis in combating acidosis? Does this not operate by substituting NH_4^+ ions to conserve fixed cations?

REPLY: Instead I prefer to say that every time an ammonium ion is substituted for a urinary urea N atom, one extra H^+ is eliminated. Again excretion of the anion excess may be considered incidental.

ANTAGONIST: But when we come to balance studies, must we not discuss acid-base regulation in terms of fixed anions and cations? The organism has an almost unlimited ability to synthesize hydrogen ions, for example by converting palmitic acid into 8 moles of acetic acid, or glucose into 2 moles of lactic acid. Surely one cannot construct a balance of H^+!

REPLY: True, the organism can form huge quantities of H^+, whether we choose to say glucose has formed lactic acid or lactate ions plus hydrogen ions. But let us complete these metabolic reactions: In order to oxidize lactate or acetate to CO_2 and water we must again take up the H^+ from the environment:

$$CH_3COO^- + H^+ + 2O_2 \rightarrow 2H_2O + 2CO_2\uparrow.$$

(The course of the reaction is of course more complex than this but the net effect is the same.) Metabolic reactions are constantly forming and removing H^+, but for such substrates the balance will be precisely zero. Only when intermediates accumulate will hydrogen ions accumulate. Subsequent catabolism of the intermediates will correct the acidosis unless, of course, the intermediates meanwhile are lost from the body, as in ketonuria, leaving a deserted hydrogen ion. Similarly, the hydrogen ions arising from metabolic CO_2 are again removed as the CO_2 is expired. Only the hydrogen ions deserted by HCO_3^- escaping into excreta will cause net acidification.

Furthermore, valuable information has already been gained by the technic of hydrogen-ion balance. For example, a negative hydrogen-ion balance (increased urinary titratable acidity and NH_4^+ excretion, decreased HCO_3^- excretion) during recovery from hypokalemic acidosis has shown that the apparently alkalotic cat actually contained *more* than a normal supply of H^+, presumably in the cells [*4*].

ANTAGONIST: How can you explain the acidosis caused by phosphate and sulfate retention, for example in uremia, except by bicarbonate displacement? Does not acetoacetate cause acidosis by displacing bicarbonate?

REPLY: How can bicarbonate be displaced unless H^+ is supplied to convert it to H_2CO_3? I question whether the mere retention of sulfate or phosphate actually is acidifying! Here, too, the hydrogen ion must cause the acidosis. In ketosis, for each acetoacetate one H^+ is formed; surely this produces the acidosis.

We must keep distinct from this problem the separate question of how acid is produced when protein is catabolized. The oxidation of a "neutral" S of cysteine or methionine to sulfate inevitably produces 2 H^+, in the test tube or in the body. It is not likely that phosphate residues release hydrogen ions upon the catabolism of phosphoproteins or other phosphomonoesters, although catabolism of such phospho*diesters* as the nucleic acids and phospholipids should lead to H^+ release. But this is a separate problem.

ANTAGONIST: You say that Na^+ and K^+ and Cl^- do not enter into reactions to produce H^+ or OH^-. Yet when Na^+ replaces cellular K^+ in potassium deficiency, is there not evidence that H^+ also accumulates in the cells? Is the Na^+ really as inert as you say?

REPLY: There must indeed be at least one exception to the biologic inertness of sodium and potassium and probably of chloride also. A carrier substance must bind Na^+ specifically to

[4] COOKE, R. E., SEGAR, W. E., REED, C., ETZWILER, D. D., VITA, M., BRUSILOW, S. and DARROW, D. C. The role of potassium in the prevention of alkalosis. *Am. J. Med.*, 17: 180, 1954.

ferry it out of the cells; the Na^+ may very well displace a H^+ in combining with the carrier. The amount of Na^+ so bound at any instant must be very small. Probably we have a valuable clue to the nature of this reaction in the curious H^+ distribution of potassium deficiency.

Note, however, that the existence of such reactions by no means justifies our considering the various fixed cations as equivalent alkalinizing agents.

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Clinical Studies

Primary Pulmonary Hypertension*

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PULMONARY hypertension has been the subject of considerable investigation since the introduction of cardiac catheterization, through which its presence can be unequivocally established. There is an extensive and expanding literature on various facets of this interesting abnormality, but many aspects of its pathologic physiology and pathogenesis remain obscure. It is now generally appreciated that pulmonary hypertension may be a concomitant or a result of a wide variety of disorders [*1,2*]. (Table I.) Many cases of so-called secondary pulmonary hypertension are commonly encountered in practically every catheterization laboratory. In most instances the associated or causative lesion can be identified, either clinically or with the aid of cardiac catheterization.

There is, however, a form of pulmonary hypertension in which one is unable to incriminate any obvious etiologic factor clinically even after careful scrutiny. Such cases have been considered by definition to represent instances of primary pulmonary hypertension. With the widespread application of cardiac catheterization, more and more cases of this type are being encountered and reported in the literature [*3–8*]. In many instances these have been reported merely as tabulations in over-all catheterization experience without detailed case reports and without autopsy confirmation [*9–11*]. This has led to a seemingly increasing incidence of a condition which previously was considered rare and diagnosable only by postmortem examination. Whether or not this increased incidence of primary pulmonary hypertension, as indicated by current statistics, is apparent or real can be established only after all these catheter and clinically diagnosed cases come to autopsy, for the appreciation of its former rarity was based on the application of critical pathologic criteria to autopsy material [*12,13*].

During the course of our first decade of ex-

TABLE I
CLASSIFICATION OF CAUSES OF PULMONARY VASCULAR DISEASE

- Acute
  - Pulmonary embolism
- Subacute
  - Miliary carcinomatosis
    - Hematogenous dissemination
    - Lymphatic dissemination
- Chronic
  - Diffuse pulmonary parenchymal disease
    - Emphysema
    - Kyphoscoliosis
    - Pulmonary fibrosis
    - Pulmonary granulomatosis
  - Diffuse pulmonary vascular disease
    - Recurrent pulmonary embolism
    - Thrombosis of pulmonary artery
    - Sickle-cell anemia
    - Schistosomiasis
    - Echinococcosis
    - Arteritis
      - Thromboangiitis obliterans
      - Polyarteritis nodosa
    - Idiopathic or primary
  - Congenital heart disease
    - Atrial septal defect
    - Ventricular septal defect
    - Patent ductus arteriosus
  - Lesions of the left side of the heart
    - Mitral stenosis
    - Chronic left ventricular failure, any cause

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perience with cardiac catheterization, we have had the opportunity to study clinically and physiologically four cases of pulmonary hypertension which by subsequent autopsy examination proved to be of the primary variety, that is to say, pulmonary hypertension of unknown cause. The purpose of this report is to emphasize the difficulties that may be encountered in ruling out certain causes of secondary pulmonary hypertension, even by present-day means. Our experience with pulmonary hypertension in general suggests that although it may be possible now to make a presumptive diagnosis of primary pulmonary hypertension during life, necropsy is still an essential part of the examination.

CASE REPORTS

CASE I. M. Q., a thirty-three year old white, childless housewife, was admitted to the Peter Bent Brigham Hospital in April, 1946. She had enjoyed good health until two and a half years before admission when the first of several syncopal attacks which were not related to exertion or accompanied by chest pain or palpitations occurred. One-and-a-half years prior to admission she noted progressive dyspnea on exertion and more frequent syncopal attacks. During the nine months preceding admission, manifestations developed of cardiac decompensation with progressive dependent edema, increasing abdominal girth and engorged neck veins. These subsided on a cardiac regimen and she was referred to the hospital for cardiac evaluation.

Physical examination revealed the following: temperature, 98°F.; pulse, 102; respirations, 20; blood pressure, 120/90 mm. Hg. The patient did not appear ill and she was able to lie flat comfortably. There was no clubbing or cyanosis of the nail beds, and all signs of congestion were absent. The heart was not enlarged. P_2 was louder than A_2. There was a soft, grade 1 precordial murmur; during a Valsalva maneuver a loud, grade 4–5 systolic whistling noise was heard over the entire precordium. The cause of this noise remained obscure.

Routine urinalysis, hemogram and blood chemistries were within normal limits except for a hematocrit of 51 per cent. Roentgenograms showed a large heart with a prominent right ventricle and pulmonary artery but normal intrapulmonary branches. Electrocardiogram (standard leads only) showed marked right axis deviation with inverted T_2, T_3, suggesting right ventricular hypertrophy.

A diagnosis of pulmonary hypertension of undetermined cause was made on the basis of cardiac catheterization and the patient was discharged to the care of her referring physician. Her subsequent course was rapidly downhill; she was admitted to another hospital during the same year for cardiac exploration, constrictive pericarditis being suspected. She died on the operating table before the pericardium was exposed.

Autopsy revealed no congenital abnormalities of the heart; there were no valvular lesions. Except for atherosclerosis of the pulmonary artery and its major branches, there were no gross abnormalities of the lungs. Microscopically, the lung parenchyma was normal. Atherosclerosis was present with atrophy of the media in larger branches of the pulmonary artery. (Fig. 1.) Arteriolar thickening was also present, in some instances it was due to medial hypertrophy (Figs. 1 and 2), in others to intimal hyperplasia. (Fig. 3.) Plexiform lesions of the smaller arteries were also observed. (Fig. 4.)

CASE II. D. L., a twenty-six year old white, single, female bookkeeper, was admitted to the Peter Bent Brigham Hospital in December, 1946, because of dyspnea, chest pain and cyanosis. Her past history was negative (hay fever, but no asthma). She was well until nine months before admission, when she first experienced fatigue and exertional dyspnea followed shortly by progressive cyanosis, dizzy spells without syncope and three-pillow orthopnea. During the three months preceding admission a hacking cough developed and she had several attacks of paroxysmal nocturnal dyspnea. She was referred to the Peter Bent Brigham Hospital for cardiac catheterization.

Physical examination revealed the following: temperature, 98.2°F; pulse, 100; respirations, 30; blood pressure, 120/100 mm. Hg. The patient was markedly cyanotic and orthopneic. No clubbing of the digits was noted, and the only sign of peripheral congestion was distended, pulsating neck veins. The heart was enlarged just beyond the midclavicular line. There were no murmurs, but P_2 was widely split and louder than A_2. The remainder of the examination was normal. Vital capacity was 3,100 cc.; circulation time, thirteen seconds; hematocrit, 56 per cent; venous pressure, 16 cm. water. X-rays of the chest showed 5 per cent transverse enlargement with a prominent pulmonary artery and pulsating, large intrapulmonary vessels as well. Electrocardiogram showed right axis deviation, biphasic T_2, and an inverted T_3, suggestive of right ventricular hypertrophy.

Cardiac catheterization revealed pulmonary hypertension and a diagnosis of pulmonary vascular disease of unknown cause was made. The patient returned to her home; she died in July, 1948, after a downhill course. A postmortem examination was performed in another hospital, and a complete report plus microscopic sections were kindly sent to us by Dr. Chester M. Kurtz.

At autopsy, on macroscopic examination, the lungs were normal except for advanced atherosclerosis of the pulmonary arteries. The heart was moderately enlarged, weighing 360 gm. There were no congenital malformations and the valves were normal. The

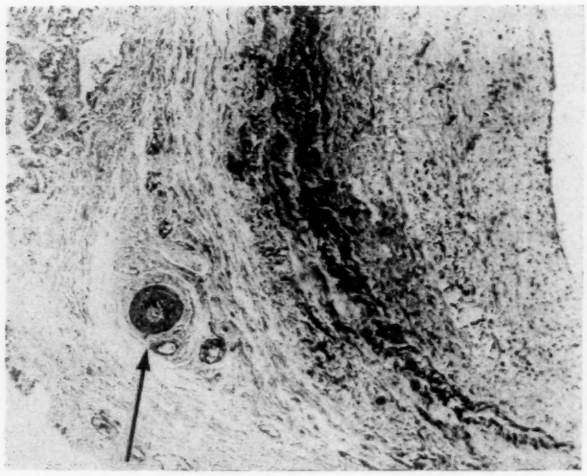

FIG. 1. On the right is a large branch of the pulmonary artery with thickened intima containing atheromatous deposits and atrophy of the media. On the left, the arrow points to a small artery exhibiting hypertrophy of the media and minimal thickening of the intima. Trichrome; original magnification, × 160. (Case I.)

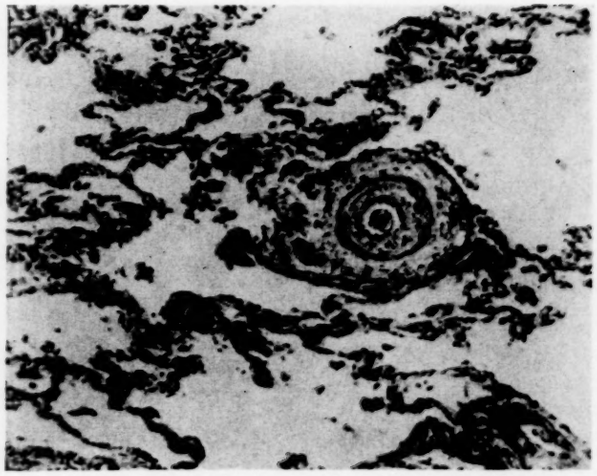

FIG. 2. The artery resembles that in Figure 1 in that there is hypertrophy of the media, but there is no intimal thickening. The alveolar walls are normal. Trichrome; original magnification, × 275. (Case I.)

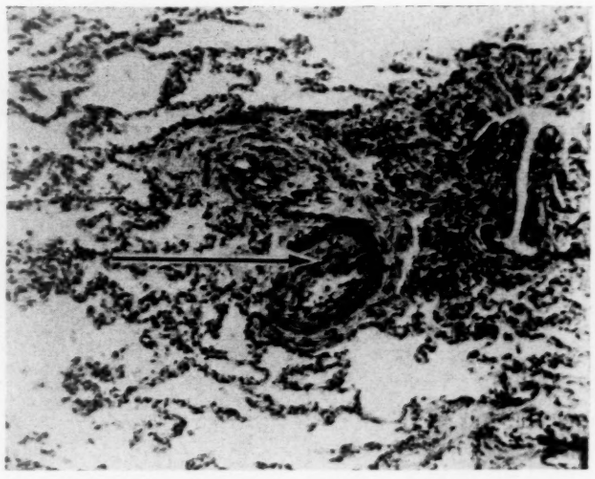

FIG. 3. On the right is a normal bronchiole. There is intimal thickening of the small artery in the center of the field, as indicated by the arrow. The alveolar walls are normal. Trichrome; original magnification, × 275. (Case I.)

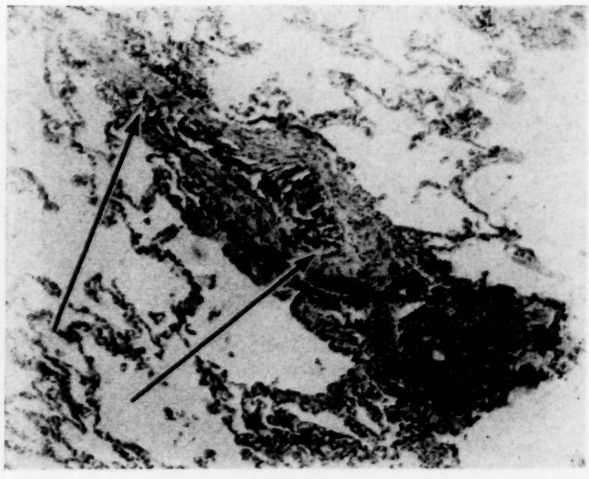

FIG. 4. Plexiform lesion involving artery which is seen in longitudinal section (limits of lesion are indicated by arrows). Trichrome; original magnification, × 160. (Case I.)

right ventricular wall was markedly hypertrophied, measuring up to 1 cm. in thickness, and the right atrium was dilated. The coronary arteries were normal, as was the pericardium.

Microscopically, there was marked thickening of the walls of the pulmonary arterioles and smaller arteries with reduction in lumen size. In some, this was accounted for by a plexiform lesion (Fig. 5); in others, there was intimal hyperplasia with a concentric pattern of laminations and a centrally located narrowed lumen. (Fig. 6.) There was slight thickening and increased cellularity of the alveolar walls. In one section there was an acute localized bronchitis, but no other evidence of infection. Distended alveoli were noted in some of the sections. The walls of the larger branches of the pulmonary artery were thickened with degenerative changes in intima and media. No other microscopic observations were pertinent. The major anatomic diagnoses were pulmonary arteriosclerosis and arteriolosclerosis, right ventricular hypertrophy and congestive failure.

CASE III. C. S., a thirty-three year old housewife, was admitted to the Peter Bent Brigham Hospital in January, 1951. She had always enjoyed good health and went through two pregnancies without incident.

FIG. 5. Artery with thickened wall and plexiform lesion. The apparent original lumen is narrowed and there are several intramural vascular spaces (see arrows). Hematoxylin and eosin; original magnification, × 400. (Case II.)

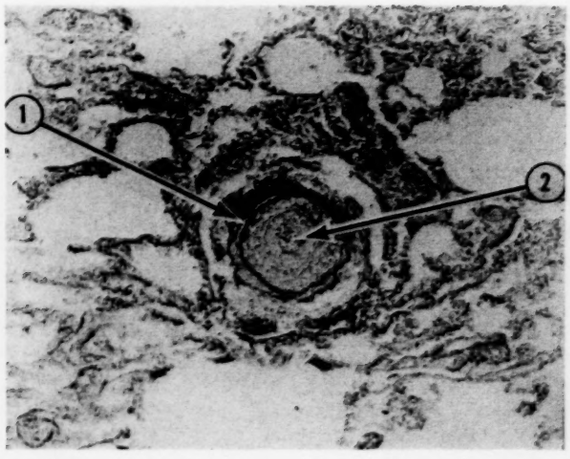

FIG. 6. Artery with advanced intimal thickening and narrowing of the lumen. Arrow 1 points to the elastica, arrow 2 to the narrowed lumen. Elastic tissue stain; no counterstain, original magnification, × 250. (Case II.)

Eight years prior to admission she first noted episodes of cyanosis of her lips and nail beds brought on by exposure to cold or exertion. One year later she spontaneously aborted during the seventh month of her third pregnancy. Thereafter she fatigued easily and became more and more dyspneic on exertion. She completed her fourth pregnancy but only after considerable difficulty with severe dyspnea, cough and dependent edema. This pregnancy left her severely limited by dyspnea, fatigue and orthopnea; by this time cyanotic episodes were quite frequent. She was examined by a cardiologist who, on the basis of cardiac enlargement and electrocardiographic evidence of right ventricular hypertrophy, believed she had a congenital cardiac defect and referred her to the hospital for further investigation.

Physical examination revealed the following: temperature, 99°F.; pulse, 100; respirations, 20; blood pressure, 100/60 mm. Hg. The patient was able to lie flat although with some respiratory distress. Cyanosis was not detectable and the nails were not clubbed. No objective signs of pulmonary or peripheral congestion were noted. The left anterior chest was more prominent than the right with a prominent precordial heave. Pulmonic valve closure was palpable in the second left intercostal space but there were no thrills. Auscultation revealed a grade 3 rumbling presystolic murmur at the apex and an early decrescendo diastolic blow along the left sternal border. P_2 was markedly accentuated.

Routine blood and urine examinations were normal except for a hematocrit of 50 per cent. Venous pressure was 13.5 cm. water; circulation time, twenty seconds; vital capacity, 2,800 cc. Chest fluoroscopy and roentgenograms showed moderate cardiac enlargement with a markedly enlarged main pulmonary artery segment and moderately enlarged and pulsating hilar vessels. The right ventricle was prominent but the left atrium did not displace the esophagus. A twelve-lead electrocardiogram was characteristic of right ventricular hypertrophy and in addition P wave changes ("P pulmonale") suggested right atrial hypertrophy.

A diagnosis of Eisenmenger's syndrome was made on the basis of cardiac catheterization and the patient was discharged to the care of her private physician. After a progressively downhill course, she was admitted to another hospital in October, 1952, in marked peripheral failure and cyanosis with severe dyspnea, weakness and orthopnea. She died in the hospital and an autopsy was performed by Dr. Irving Goodof, who was kind enough to send us his report and microscopic sections of the lungs.

The major anatomic diagnoses included arteriosclerosis and arteriolosclerosis of the pulmonary arteries, acute (Fig. 7) and subacute arteritis of the pulmonary arteries with recent and partially organized thrombi, right ventricular hypertrophy and dilatation, and chronic passive congestion of the lungs, liver, spleen and kidneys. There were no congenital abnormalities or valvular lesions of the heart. No other gross or microscopic alterations were pertinent.

CASE IV. C. L., a forty-two year old mother of seven children, was admitted to the Peter Bent Brigham Hospital in June, 1950. The patient had unexplained fainting spells during childhood but thereafter led a vigorous life which included seven normal pregnancies and full-time work as well as all her own housekeeping. Two years before admission she began having progressive exertional dyspnea, fatigue and cyanotic episodes. In addition, she occasionally experienced a precordial aching pain on exertion which radiated to the back. Her chief complaints were increasing fatigue and lack of energy. She was hospitalized elsewhere and a tentative diagnosis of atrial

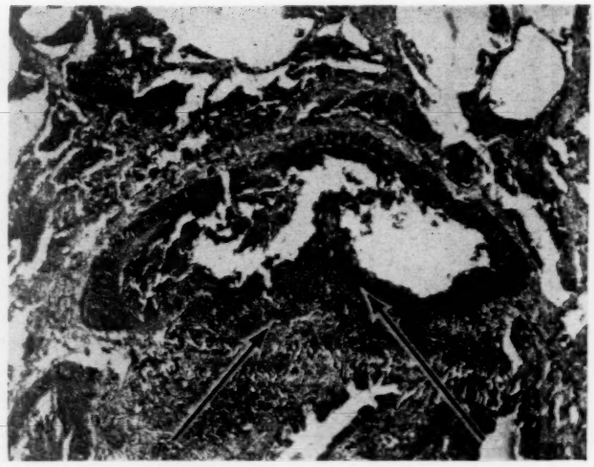

FIG. 7. Acute arteritis. Note the normal portion of the pulmonary artery in the upper half of the field. The necrosis and cellular reaction in the lower half are identified by arrows. Hematoxylin and eosin; original magnification, × 125. (Case III.)

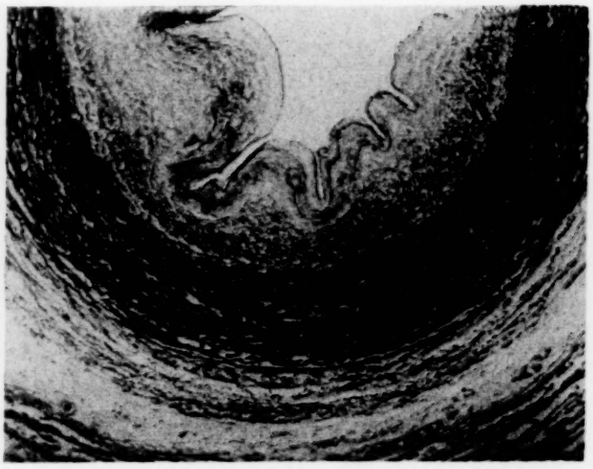

FIG. 8. Major branch of pulmonary artery with uniform intimal thickening and atheromatosis. Verhoeff-Van Gieson elastic tissue stain; original magnification, × 125. (Case IV.)

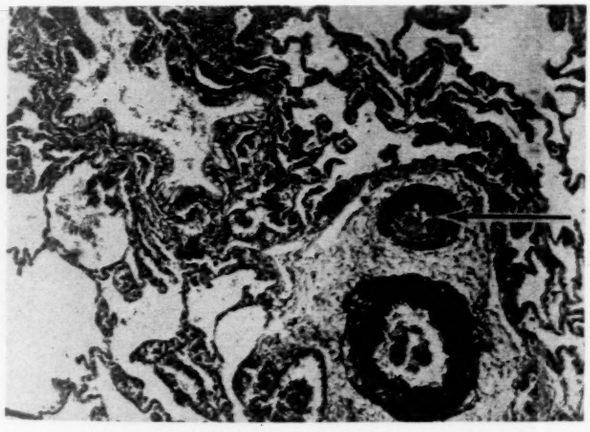

FIG. 9. The bronchiole at the upper left and the alveolar walls are essentially normal. The larger artery at the lower right is normal but the smaller artery above has advanced intimal thickening (see arrow) with narrowing of the lumen. Verhoeff-Van Gieson elastic tissue stain; original magnification, × 200. (Case IV.)

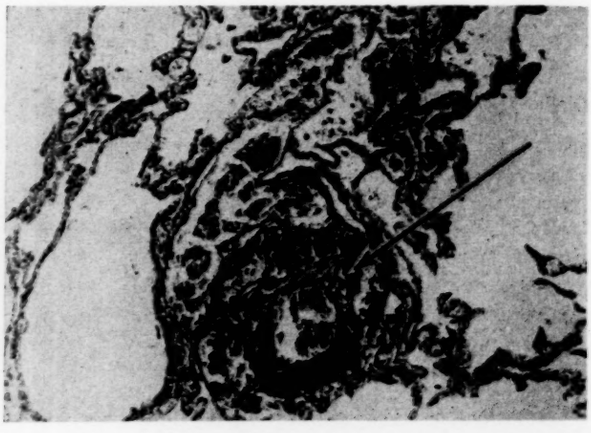

FIG. 10. Artery below center of field shows attenuation and loss of elastica and muscularis with replacement by multiple vascular channels (see arrow) which communicate with sinusoid-like formation in upper half of field. Verhoeff-Van Gieson elastic tissue stain; original magnification, × 200. (Case IV.)

septal defect was made. Subsequently she was referred to the Peter Bent Brigham Hospital for cardiac catheterization.

Physical examination revealed the following: temperature, 99°F.; pulse, 84; respirations, 20; blood pressure, 125/80 mm. Hg. The patient was moderately obese with a plethoric facies and slightly cyanosed lips and nails but no clubbing. The remainder of the physical examination, including the heart, was within normal limits. There were no cardiac murmurs and the pulmonary second sound was not accentuated.

Routine blood, urine and chemistries were all normal except for a hematocrit of 56 per cent. Venous pressure was 12.5 cm. water; circulation time, twenty seconds; vital capacity, 2.0 L. Chest fluoroscopy and roentgenograms showed a heart of normal transverse diameter with a prominent main pulmonary artery segment and suggestive enlargement of the right ventricle but no other abnormalities. A twelve-lead electrocardiogram was characteristic of right ventricular hypertrophy.

Cardiac catheterization revealed marked pulmonary hypertension, and a diagnosis of Eisenmenger's syndrome was considered. During the following two years she had episodes of peripheral congestion with increasing dyspnea, non-productive cough and cyanosis. She died in April, 1953. An autopsy, limited to

FIG. 11. Adjacent branches of the pulmonary artery show abnormal vascular channels without evidence of intimal hyperplasia but with apparent atrophy of the muscularis of the media. Arrow 3 indicates original lumen replaced by multiple channels which may represent a canalized thrombus. Other arrows indicate vascular channels in the media which has an altered muscularis and elastica pattern. At the upper right is a sinusoid-like formation. Verhoeff-Van Gieson elastic tissue stain; original magnification, × 400. (Case IV.)

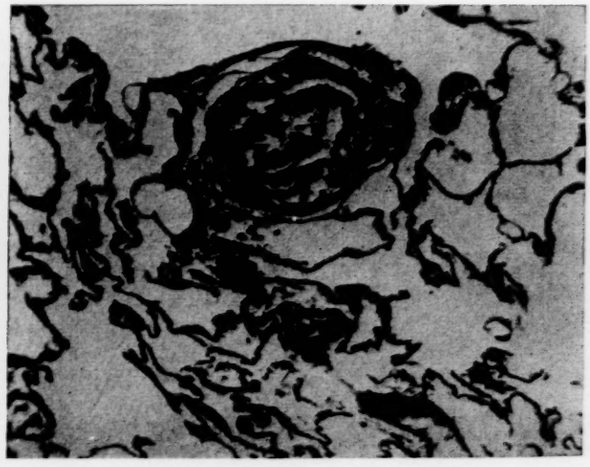

FIG. 12. Multiple vascular channels occupying the position of the media are present thus resembling Figures 10 and 11, but there is no intimal proliferation and no suggestion of remote thrombosis. The alveolar walls are normal. Verhoeff-Van Gieson elastic tissue stain; original magnification, × 200. (Case IV.)

the thorax, was performed. Microscopic slides were sent to us through the courtesy of Dr. Reginald K. House.

Postmortem examination was restricted and information was obtained only on the heart and lungs. The heart was enlarged, weighing 440 gm. There were no congenital abnormalities. The interatrial and interventricular septa were intact and the valves, pericardium and epicardium were normal. The right ventricular wall was thickened measuring 0.8 cm., indicating right ventricular hypertrophy. The pleural surfaces were normal. There was marked sclerosis of the pulmonary arteries and no other gross abnormalities.

Microscopically, the larger branches of the pulmonary artery showed uniform intimal thickening with atheromatosis. (Fig. 8.) The bronchioles and alveolar walls were normal. In smaller arteries there was intimal thickening. (Fig. 9.) In other small arteries there were degenerative alterations of the media without intimal hyperplasia but with unusual intramural vascular channels which communicated with sinusoid-like formations which ultimately could be traced to dilated alveolar capillaries. The apparent formation of accessory vascular channels intramurally was borne out by elastic tissue stains. (Figs. 10, 11 and 12.) With trichrome stains, it was evident that medial degeneration with plexiform lesions underlay these changes. (Fig. 13.) In some arteries both medial degeneration with accessory channel formation and intimal alterations suggestive of organized and canalized thrombi were present. (Figs. 11 and 14.) Other arteries showed abnormal channel formation in the absence of intimal proliferation and plexiform lesions. (Fig. 15.)

The major anatomic diagnosis on the basis of this restricted examination were hypertrophy and dilatation of the heart with right ventricular hypertrophy, pulmonary atherosclerosis and arteriolosclerosis with acquired vascular abnormalities related to medial degeneration secondary to arteriosclerosis; intimal hyperplasia, possibly secondary to thrombosis or embolization.

COMMENTS

Clinical. Table II summarizes the clinical findings in our four cases. All patients were young women. Two had had no pregnancies; two had had multiple pregnancies. The duration of symptoms from onset to death ranged from three-and-a-half to twelve years. Exertional dyspnea of a progressive nature and fatigue were prominent symptoms in all. Syncope, cyanotic episodes, chest pain, cough and edema were present less commonly and in varying combinations in each patient. Only one patient was detectably cyanotic, although three described cyanotic episodes; none had clubbing, and two showed one or more signs of peripheral congestion. Of the two patients who had heart murmurs, in one it was a nonspecific precordial systolic murmur (M. Q.) while in the other (C. S.) two distinct diastolic murmurs were heard and confirmed by phonocardiogram. The middiastolic rumble at the apex was indistinguishable from that of mitral

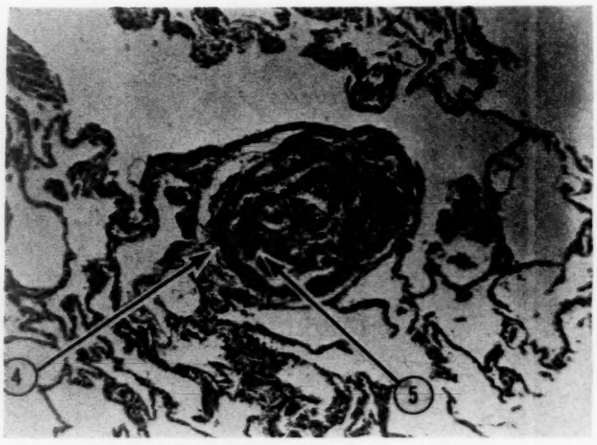

FIG. 13. This is the same artery as in Figure 12 but seen at a different level and stained to demonstrate cells and smooth muscle. The only smooth muscle present is indicated by the arrow 4 and a portion of a plexiform lesion by arrow 5. Trichrome; original magnification, × 200. (Case IV.)

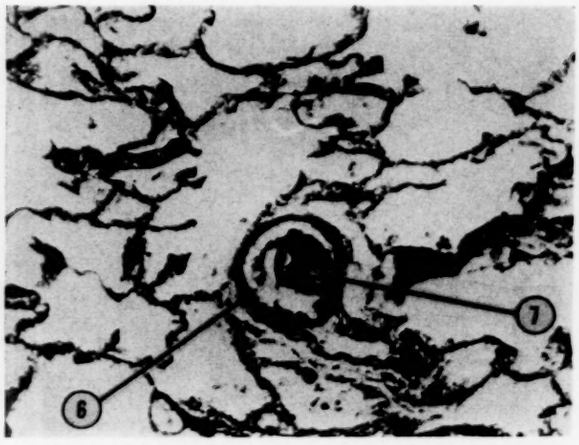

FIG. 14. In this artery, no muscularis can be identified, but a vascular channel (see arrow 6) with inner and outer walls composed of elastica occupies the position of the media. The former lumen shows intimal changes suggesting those resulting from canalization of a thrombus (see arrow 7). Verhoeff-Van Gieson elastic tissue stain; original magnification, × 200. (Case IV.)

stenosis and prompted a diagnosis of Lutembacher's syndrome by several examiners. The second diastolic murmur down the left sternal border was consistent with pulmonic insufficiency. The vital capacities in three were only slightly subnormal and moderately reduced in the fourth. Hematocrits were all slightly to moderately elevated. Twelve-lead electrocardiograms were available in only two patients and showed characteristic changes of right ventricular hypertrophy in the precordial leads, while in the two patients studied earlier, only standard limb leads were available which showed right axis deviation with ST-T changes in standard leads II and III suggesting right ventricular hypertrophy in addition. Roentgen-

TABLE II

CLINICAL FINDINGS IN PRIMARY PULMONARY HYPERTENSION

Patient	Age at Death (yr.)	Sex	Duration of Illness (yr.)	Symptoms							Physical Examination					Laboratory Examination							
				Dyspnea	Fatigue	Syncope	Chest Pain	Cyanosis	Cough	Edema	Cyanosis	Clubbing	Congestion	Heart Murmur	Pulmonic 2nd Sound	Vital Capacity (L.)	Hematocrit (%)	Electrocardiogram	X-ray				
																			Size*	Main Pulmonary Artery Prominence	Hilar Dance	Right Ventricular Enlargement	Peripheral Pulmonary Vasculature
M. Q.	33	F	4	+	+	+	0	0	0	+	0	0	0	+	+	3.1	51	RAD†	32%	++	0	+	N
D. L.	28	F	3½	+	+	+	+	+	+	0	+	0	0	0	+	3.1	56	RAD†	5%	++	+	+	N
C. S.	34	F	12	+	+	0	0	+	+	+	0	0	0	+	+++	2.8	50	RVH	31%	++	0	+	..
C. L.	45	F	4	+	+	0	+	+	0	0	±	0	0	0	±	2.0	56	RVH	0	++	0	±	N

* Per cent increase in transverse diameter, criteria of Ungerleider and Clark.

† Standard leads only, right axis deviation with ST-T changes in leads II and III, indicating probable right ventricular hypertrophy.

ographic examination revealed uniform prominence of the main pulmonary artery in all cases with typical hilar dance in one, cardiac enlargement in two, and right ventricular enlargement in all.

The clinical findings in our four cases, summarized in Table II, conform in general to those described by Dresdale et al. [*3*]. The correlation of physiologic observations with clinical manifestations has made it possible to recognize or suspect clinically the presence of pulmonary hypertension in most instances without difficulty. The combination of an enlarged right ventricle and main pulmonary artery segment without "hilar dance" by x-ray and right ventricular hypertrophy by electrocardiogram immediately suggests its presence, although similar findings occasionally may be seen in certain cases of uncomplicated atrial septal defect or pulmonic stenosis with poststenotic dilatation. The question, therefore, is whether or not there are any clinical clues which enable one to differentiate between primary and secondary forms of this abnormality. A predilection for females has been suggested, but the 60 per cent incidence cited by Dresdale is not impressive. By far, a great majority of cases have been reported in young adults, but the age, according to the same authors, ranges from childhood to old age. The important symptoms in primary pulmonary hypertension include exertional dyspnea, fatigue, cyanosis, syncopal attacks of dizziness and angina-like chest pain.

The mechanisms underlying these symptoms remain obscure. Dyspnea occurred uniformly, but usually without striking orthopnea, curtailment of vital capacity or other parameters of respiratory function [*3*]. Syncope, on the other hand, occurred in only 25 per cent of the cases collected by Dresdale. Dressler [*14*] has drawn attention to the diagnostic significance of these attacks in patients with primary pulmonary hypertension. He pointed out that such attacks also occur in patients with cyanotic congenital heart disease, but believed that differentiation of the two disorders should not prove difficult. Howarth and Lowe [*15*] studied the effect of exercise on two patients with primary pulmonary hypertension who had syncopal attacks and five patients with pulmonary hypertension associated with various congenital cardiac defects, of whom only two gave histories of syncopal attacks. They believed the syncopal attacks precipitated in the two patients with primary pulmonary hypertension could be due to acute right ventricular failure. Syncope was not induced in any of the patients with congenital heart disease, but it was suggested that a sudden decrease in arterial oxygen saturation may be a factor causing syncope in these cases.

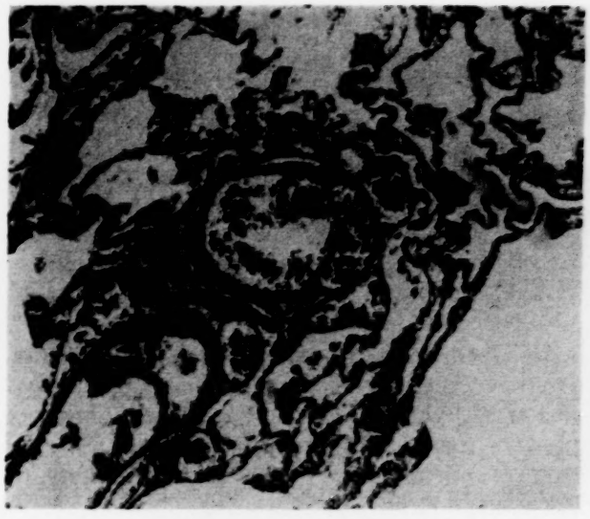

FIG. 15. Abnormal intramural vascular channels are present in these arterial branches without evidence of intimal proliferation or plexiform lesions, but with apparent loss of the muscular portion of the media. Verhoeff-Van Gieson elastic tissue stain; original magnification, × 200. (Case IV.)

The chest pain which may occur is oftentimes indistinguishable from true angina pectoris. However, its relationship to myocardial ischemia is not known, as the coronary arteries are usually free from affliction. This type of pain has come to be known as the pain of pulmonary hypertension; that it may occur in other forms of pulmonary hypertension has been unequivocally established by Viar and Harrison [*16*]. They listed the four most common underlying disorders which may produce this pain: (1) lesions of the mitral valve, usually stenotic but occasionally regurgitant; (2) primary diffuse disorders of the lungs, especially asthma, emphysema and bronchiectasis; (3) disorders of the pulmonary arteries, of which embolism is the most frequent; and (4) those congenital malformations of the heart which lead to elevation of pressure in the pulmonary artery.

Persistent or episodic cyanosis was a striking feature in the cases collected by Brenner [*12*]. Its occurrence in a patient with pulmonary hypertension immediately suggests the possibility of a congenital cardiac defect with right-to-left

shunt. However, it has been demonstrated that cyanosis (more precisely, arterial oxygen unsaturation) which is not infrequently seen in primary pulmonary hypertension may occur in the absence of such defects [*17–22*]. It is doubtful that cyanosis, when it occurs, can be ascribed to a single etiologic factor. Cases have been reported wherein an anatomic and/or physiologic patent foramen ovale was found at autopsy [*7,10,23,24*]. With the elevation of right atrial pressure commonly present in these cases, it is easily conceivable that an unsealed foramen could become functional. Such an explanation is undoubtedly true in others, but in the majority of cases the foramen is found to be sealed and other factors, particularly pulmonary diffusion or shunt, must have been operating.

The physical, electrocardiographic and roentgenographic manifestations listed in Table II are uniformly present and are characteristic reflections of moderate to severe degrees of pulmonary hypertension. Unfortunately, they have no conclusive significance as to the nature of the pulmonary hypertension, as identical findings are seen in most forms of secondary pulmonary hypertension. Murmurs of variable location, timing and characteristics may be heard in primary pulmonary hypertension, and this fact has often prompted an erroneous diagnosis of congenital heart disease, particularly in patients who are also cyanotic. The Graham-Steell murmur of relative pulmonic insufficiency is commonly present and its occurrence is understandable, but no attempt has been made to explain the various other murmurs that have been described.

Brill and Krygier [*13*] were the first to point out the uniformity in the clinical picture of primary pulmonary hypertension. More recently, Dresdale et al. [*23*] have pointed out that the syndrome is sufficiently distinct that it is possible to recognize it clinically. It is apparent from the clinical data that our cases conformed to this uniform profile; however, we have a somewhat more pessimistic view towards the possibility of making the diagnosis on clinical grounds alone or, in some cases, even with the aid of cardiac catheterization. It is highly unlikely on theoretic grounds that there is anything specific about these manifestations in primary pulmonary hypertension, considering the complexity of their genesis, and as we have pointed out any or all are known to occur in patients with secondary pulmonary hypertension. We have been impressed with the difficulty of identifying the rare individual case of primary pulmonary hypertension against the background of cases of pulmonary hypertension from specific causes. It is our opinion, therefore, that although these patients may present a fairly uniform picture, it is not sufficiently specific to enable the diagnosis to be made clinically. The recent observations of Cutler et al. [*24*] lend considerable support to this view. These authors described a group of patients with pulmonary hypertension who presented a uniform clinical picture which is virtually indistinguishable from the supposedly characteristic picture of primary pulmonary hypertension suggested by Dresdale. They introduced the term "pulmonary vascular obstruction syndrome" for this etiologically heterogeneous group in which the common denominator appeared to be an increased pulmonary vascular resistance sufficient to result in pulmonary artery pressures of systemic magnitude. The autopsy protocol of their Case I and catheterization data in five patients indicate that most of these patients had congenital cardiac defects, most notably Eisenmenger's syndrome. The findings in the second autopsied case are compatible with primary pulmonary hypertension with a patent foramen ovale. The authors pointed out the difficulties that may be encountered in detecting clinically the associated congenital defect and suggested that this characteristic syndrome developed irrespective of the presence or absence of such malformations. This is a possibility which previously had not been explored, but such cases by definition are not considered examples of primary pulmonary hypertension.

The introduction of cardiac catheterization has helped immeasurably in the solution of many aspects of this clinical diagnostic dilemma. In addition it has dispelled any doubts that may have existed concerning the role played by pulmonary hypertension as the cause of isolated right ventricular hypertrophy [*18*]. It has also elucidated the pathologic physiology of this disorder.

Cardiac Catheterization. Table III summarizes the salient features of cardiac catheterization in our four cases. The most striking abnormalities in all patients were the elevated pulmonary artery pressures and calculated vascular resistance. Arterial oxygen saturations were below normal in all, but in only one was it of a degree sufficient to produce clinically detectable cyano-

TABLE III
HEMODYNAMIC FINDINGS IN PRIMARY PULMONARY HYPERTENSION

Patient	Date	Body Surface Area (sq. meter)	Brachial Artery			O_2 Consumption (cc./min.)	A-V O_2 Difference (cc./L.)	Cardiac Output (L./min.)	Cardiac Index (L./min./M²)	Pressures (mm. Hg)						Resistances (dynes sec. cm.$^{-5}$)		
			O_2 Content (vol. %)	O_2 Capacity (vol. %)	O_2 Saturation (%)					Brachial Artery		Pulmonary Artery		Right Auricle, Mean	Pulmonary "Capillary," Mean	Total Systemic	Total Pulmonary	Pulmonary Vascular
										Systolic/Diastolic	Mean	Systolic/Diastolic	Mean					
M. Q.	4/26/46	1.53	19.4	20.9	93	184	62	3.0	1.9	.....	..	80/55	67	5	..	....	1787	....
D. L.	12/13/46	1.73	18.6	24.2	77	190	54	3.5	2.0	119/77	94	61/31	46	−7	3	2149	1051	983
C. S.	1/18/51	1.64	20.8	21.9	95	217	90	2.7	1.6	110/70	90	108/56	74	11	..	2667	2193	....
C. L.	6/14/50	1.61	21.3	23.8	89	327	97	4.5	2.8	128/88	98	112/50	73	10	5	1742	1298	1209

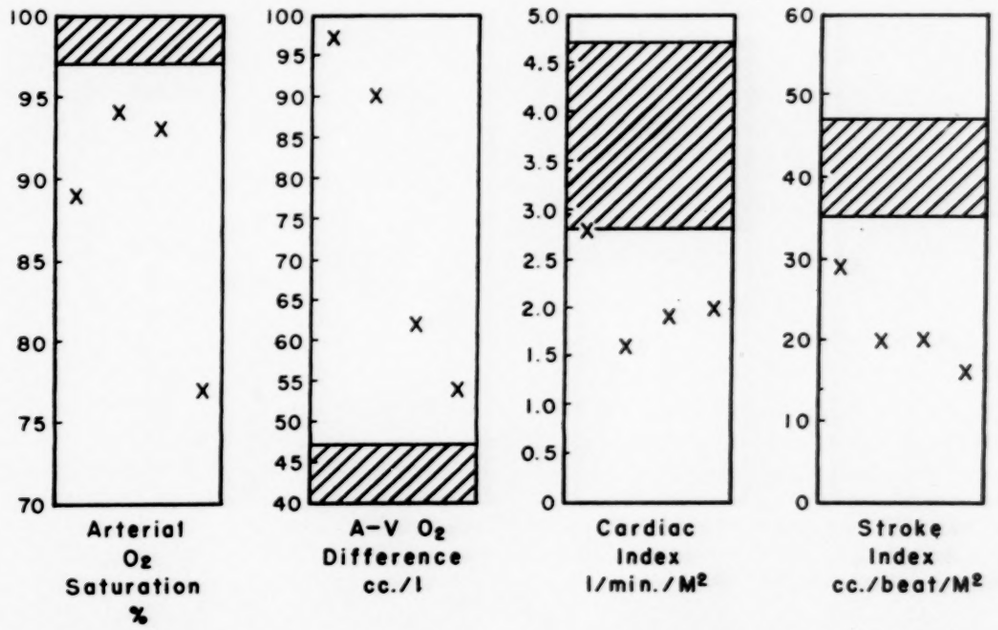

FIG. 16. Hemodynamic changes in primary pulmonary hypertension. The cross-hatched areas represent the normal ranges. The crosses are the values in our four cases. See text for discussion.

sis. Cardiac minute outputs were reduced. Two patients had elevated mean right atrial and right ventricular diastolic pressures consistent with right ventricular failure and with the clinical evidences of peripheral congestion. Valid pulmonary "capillary" pressures were obtained in only two cases; in each instance it was low-normal. These abnormalities are more easily appreciated in the graphic representations in Figures 16 to 18. The near identity of pulmonary artery and systemic artery pressures in two cases (C. S. and C. L.) suggested the physiologic abnormalities seen in Eisenmenger's syndrome, that is, functional communication of the ven-

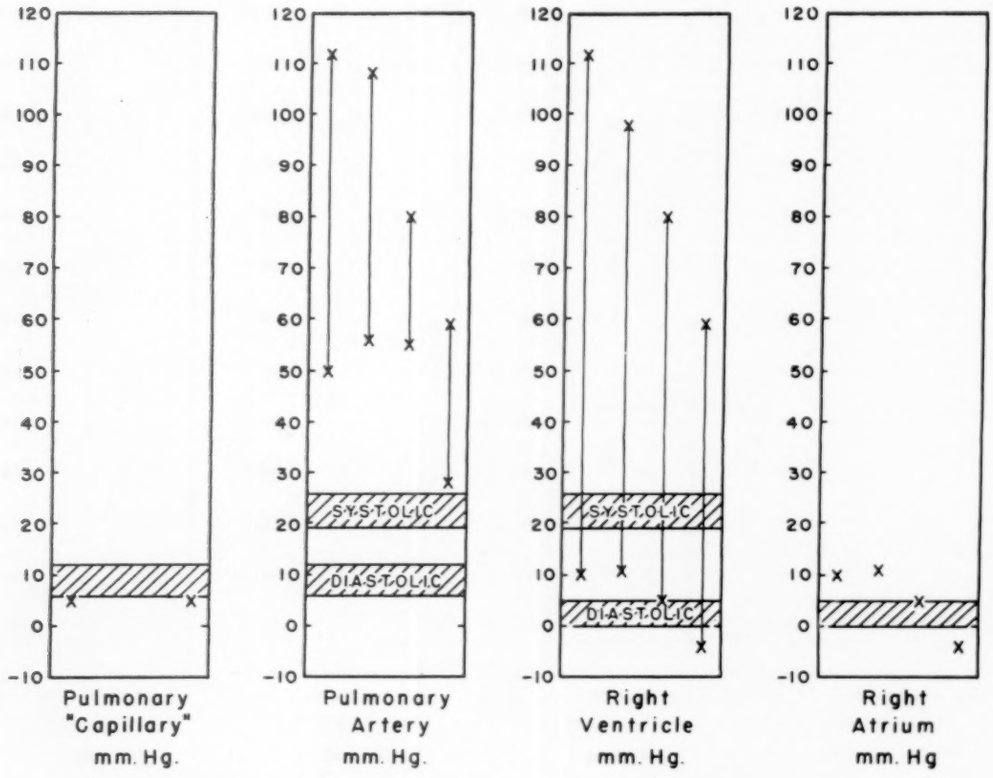

FIG. 17. Hemodynamic changes in primary pulmonary hypertension. The cross-hatched areas represent the normal ranges. The crosses are the values in our four cases. See text for discussion.

tricles sufficient to produce identity of systolic pressures, even though there were no demonstrable left-to-right shunts. The mild arterial unsaturation in one case (C. L.) was completely alleviated by inhalation of 100 per cent oxygen for fifteen minutes, indicating either that pulmonary rather than shunt factors were operating to produce it or that a right-to-left shunt had been eliminated by breathing oxygen [*25*].

The ultimate physiologic defect which results in precapillary pulmonary hypertension, whether primary or secondary, is the great increase in resistance to blood flow across the pulmonary bed, as shown diagrammatically in Figure 18. That this intrapulmonary stenosis resides in the smaller pulmonary arteries proximal to the capillary bed is clearly demonstrated by the high pressure gradient between the pulmonary artery and pulmonary artery wedge pressure (D. L. and C. L.). Figure 19 illustrates schematically the disturbed physiology of this condition. The high resistance in the pulmonary vasculature appears to reside in the arterioles and small arteries, resulting in high pressures proximally (that is, in the right ventricle and pulmonary artery) and a decreased flow (cardiac output). Whether the pulmonary vascular disease is primary, as in these four cases, or secondary to the various diseases shown in Table 1, the circulatory derangements are identical, providing there is no arterial oxygen unsaturation.

The pathogenesis of the increased pulmonary vascular resistance in our four cases remains completely obscure; whether the variable vascular changes are primary or secondary is yet to be solved, and the role played by thrombosis is controversial. As discussed later under "Pathology" there is more support for the concept that the vascular lesions are secondary to the pulmonary hypertension. Physiologic studies and clinical observations in various forms of pulmonary hypertension indicate that this increased resistance may not be as fixed and irreversible as the pathologic lesions would indicate, and that a variable component of pulmonary arterial vasomotor activity contributing to the resistance can be inferred [*23,25–32*]. Dresdale and co-workers have studied the same

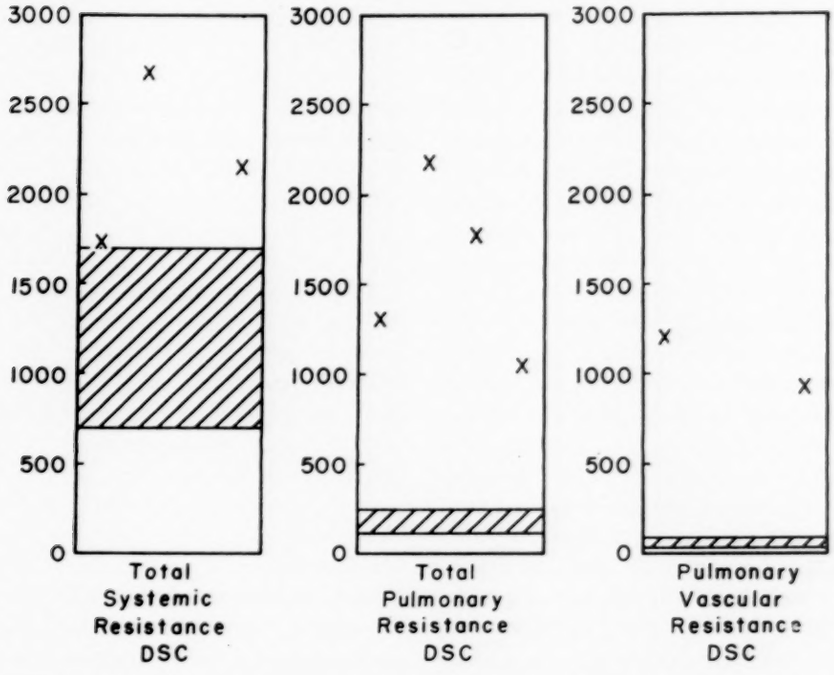

FIG. 18. Hemodynamic changes in primary pulmonary hypertension. The cross-hatched areas represent the normal ranges. The crosses are the values in our four cases. DSC refers to the resistance units, dynes seconds cm.$^{-5}$. See text for discussion.

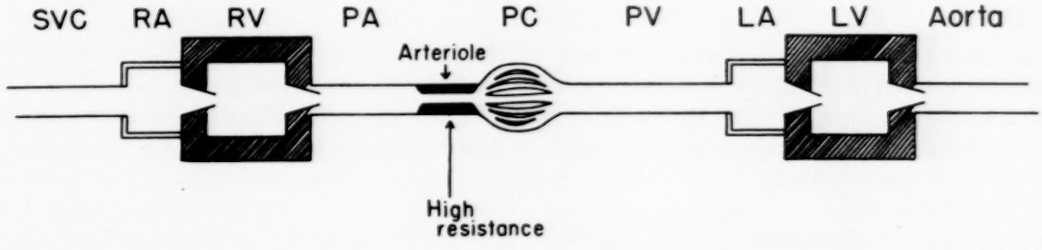

FIG. 19. Schema of the circulation in pulmonary vascular disease. Whether or not the condition is primary or secondary to a known process (see Table 1), there is an increased resistance to blood flow through the precapillary areas of the lung producing an elevation of pressures proximal to this point and a reduction of cardiac output.

problem in patients with primary pulmonary hypertension [*23*]. From their data on the effects of priscoline,® they suggested that an overactive autonomic nervous system might also be a contributory factor in this disorder. Further evidence that there is, at least in certain cases, an element of increased vascular tone is suggested by the rare cases in which the existence of pulmonary hypertension during life was substantiated by cardiac catheterization, in which subsequent postmortem examination revealed no demonstrable abnormalities in the pulmonary vasculature [*8,18*]. Similar cases were previously reported under terms which cast considerable doubt on the role played by pulmonary hypertension as the cause of isolated right ventricular hypertrophy but in retrospect probably represent forms of primary pulmonary hypertension.

It is possible to exclude by cardiac catheterization most cases of pulmonary hypertension due to congenital heart disease or obstructing lesions in the left side of the heart because of demonstrable left-to-right shunts or elevated pulmonary capillary pressures. However, there are lesions which, under certain circumstances, may prove extremely difficult and deserve particular attention. Differentiation by cardiac catheterization of recurrent "silent" pulmonary emboli or

thromboses from primary pulmonary hypertension is impossible [*33*]. This is not surprising considering that even at autopsy a variable interpretation is given to the occlusive pulmonary vascular lesions which may be seen in practically all forms of pulmonary hypertension. Barnard [*34*] has recently discussed the possible interrelationship between cor pulmonale of recurrent pulmonary thromboembolism and primary pulmonary hypertension, stating, "It is not unlikely that some cases recorded as primary pulmonary hypertension might have been due to chronic thromboembolism because organizing blood clots were regarded as secondary to the arteriosclerosis." Whether such lesions, present in a more or less minor degree in primary pulmonary hypertension, play a primary role or represent secondary concomitants of preexisting pulmonary hypertension has been the subject of considerable speculation, but remains unsolved. Suffice it to say that information afforded by cardiac catheterization falls far short of solving this pathologic difficulty. It is well known that the development of pulmonary vascular disease not infrequently complicates the course of certain forms of congenital cardiac defects with predominant left-to-right shunts, and when it is of sufficient degree it may cause abolition or even complete reversal of the shunt [*35*]. In patent ductus arteriosus, aortic-pulmonary fenestration, and ventricular septal defect (including Eisenmenger's syndrome) this situation results when pulmonary resistance equals or exceeds systemic resistance [*36*] and in atrial septal defect when the right ventricle fails, usually as a consequence of the development of pulmonary hypertension and is no longer capable of a supernormal output [*37*]. In most such cases, left-to-right shunts, even though of small magnitude, can still be demonstrated, but in rare cases the left-to-right shunts may be undetectable by the usual means or in fact completely abolished. In this situation characteristic murmurs may disappear and hemodynamic alterations indistinguishable from primary pulmonary hypertension with or without cyanosis evolve. Eisenmenger's syndrome may be suggested by identical systemic and pulmonary arterial pressures [*38*]. If this occurs in primary pulmonary hypertension, as it did in two of our cases, it is merely fortuitous, while in Eisenmenger's syndrome it is the result of a physiologically common ventricle. We believe these two possibilities can be separated by differentially altering either the pulmonary vascular resistance, by breathing low oxygen mixtures, or systemic vascular resistance by exercise or peripheral vasodilators or vasoconstrictors. Such maneuvers will produce no beat-for-beat differences of systolic pressure in the pulmonary artery and brachial artery recorded simultaneously if there is a physiologically common ventricle, whereas in all other conditions including primary pulmonary hypertension divergences will occur. As described earlier, arterial oxygen saturations before and after fifteen-minute breathing of 100 per cent oxygen will serve in most cases to differentiate right-to-left shunt factors from pulmonary diffusion factors as the cause of cyanosis, but they will not indicate at what level the shunt is occurring. Identification and localization of right-to-left shunts is now possible by the analysis of continuously recorded arterial dye dilution curves obtained after injections distal to and at or proximal to the site of the shunt, as described by Swan and Wood [*39*].

The importance of obtaining pulmonary "capillary" pressures in patients with pulmonary hypertension should be stressed. We have encountered patients with pulmonary hypertension due to mitral stenosis in whom the typical diastolic murmur and left atrial enlargement may be minimal or absent, thus clinically and hemodynamically indistinguishable from primary pulmonary hypertension except for an elevated pulmonary capillary pressure. Therefore, it is only by this measurement that it is possible to differentiate this postcapillary form of pulmonary hypertension from precapillary forms, such as primary pulmonary hypertension. It should be pointed out, however, that valid pulmonary capillary pressures are most difficult to obtain in patients with pulmonary vascular disease [*40*].

From the foregoing considerations, it is apparent that there is no single feature or combination of features in either the clinical or catheterization evaluation that can be considered as characteristic of primary pulmonary hypertension. However, a diagnosis can be made by painstakingly excluding all factors which would result in secondary pulmonary hypertension. Even thus derived, the possibility exists that subsequent postmortem examination may not corroborate the antemortem diagnosis. Whether or not this will be the case in the increasing number of cases diagnosed antemortem remains to be seen. On

the other hand, it is evident from case reports in the literature that patients diagnosed clinically as having secondary pulmonary hypertension may be proved to have primary pulmonary hypertension at postmortem. In our series two patients (C. S. and C. L.) were thought to have Eisenmenger's syndrome; the first two autopsied patients of Soulié et al. [*4*] were diagnosed clinically as having atrial septal defect and mitral stenosis respectively; another patient [*41*] recently encountered at the Boston Lying-In-Hospital was, during life, considered to have a congenital cardiac defect; the patient died suddenly during the postpartum period and was found at autopsy to have recurrent pulmonary emboli. In our opinion, therefore, necropsy is still essential in the examination of these cases. Without necropsy, the diagnosis should be considered only a presumptive one, and in the patient's best interest efforts to identify a lesion that could be treated should not be abandoned.

Pathology. In summarizing the autopsy observations in these four cases, it is apparent from the data available that only two lesions are common to all. These are the arteriosclerosis and arteriolosclerosis of the pulmonary arteries. Both lesions can be regarded as the sequelae of pulmonary hypertension. In addition to their morphologic features, which support this, these lesions were observed in women presenting no atherosclerosis of the systemic arteries, who were not diabetic and whose average age was thirty-six years, with a range of twenty-six to forty-two years.

With reference to the arteriosclerosis, the intima throughout shows varying degrees of alteration from slight thickening with sparse cellularity to marked thickening with atheromatous deposits. In these cases, no stages were found which would identify this lesion as an organized thrombus. It is recognized that the organized thrombus having its origin in an embolus can resemble the arteriosclerotic plaque, but the possibility remains that hypertension may, directly or indirectly, result in intimal alterations ranging from slight proliferation and thickening to more profound changes which may become the sites of autochthonous thrombosis as observed in the aorta. The lesion characteristic of those in this group of cases is shown in Figure 8. In the rabbit, the intravenous injection of clotted blood has resulted in arteriosclerotic lesions in the pulmonary arteries [*42,43*]. These "arteriosclerotic lesions are actually organized thrombi" and are not considered to be the "result of pulmonary hypertension of other hemodynamic alterations associated with obstruction" [*43*].

Intimal thickening in the smaller arteries and arterioles with narrowing of the lumen, as noted in these cases, is generally accepted as a consequence of pulmonary hypertension and occurs with regularity irrespective of the lesion producing the pulmonary hypertension.

In three of the four cases, plexiform lesions were present in the arteries. From step sections done on Case IV, we concluded that this lesion resulted from endothelial proliferation related to mural degeneration. Alterations in the elastica and muscularis were noted as well as abnormal vascular communications with the alveolar capillaries. (Figs. 10, 11, 12 and 13.) This is in agreement with the studies of Edwards [*44*] and Liebow [*46*]. We also share their view that these lesions are not organized thrombi. In Figure 14, the association of a canalized thrombus with some of the changes just described is demonstrated. The vascular communications resulting from these changes are arterial-capillary. We have observed similar lesions in the lungs in advanced mitral stenosis. Such lesions have been described as glomus tumors, glomus-like lesions and arteriovenous communications. The plexiform lesion appears to be the result of endothelial proliferation and consists essentially of endothelial cells. This lesion has been noted in arteries in which there may be little intimal thickening but in which there is often evidence of medial degeneration. These are the major features which distinguish it from the canalized thrombus which has, between its vascular channels and the media, partially organized thrombus which in its ultimate stage is represented by a relatively acellular tissue. Canalized thrombi, furthermore, when they are observed in the pulmonary arteries, are often found in arteries in which the media is essentially normal.

In one of the four cases (Case I, M. Q.) there was medial hypertrophy of the small arteries and arterioles. This has been regarded by some as the basis for primary pulmonary hypertension and a persistence of the thickened media normally present in fetal life [*47*]. That medial hypertrophy can be acquired is described by Edwards [*44*]. It seems entirely plausible, as postulated by others, that medial hypertrophy is an initial response to pulmonary hypertension, followed

later by the changes described in the two paragraphs preceding.

In Case III (C. S.) the unique lesion was the acute arteritis. In some sections this appeared subacute and some arteries had narrowing of the lumen produced by thrombi, which were canalized and partially organized. It is possible that arterial lesions may be the result of pulmonary hypertension, as described by Edwards [*44,45*], Liebow [*46*] and Spain [*49*]. The opposite relationship is suggested by Braunstein [*48*], who described six cases as pulmonary "periarteritis nodosa" with pulmonary hypertension. Three patients had advanced mitral stenosis, one had an interatrial septal defect, one had multiple thrombotic occlusions of the small arteries and the sixth was described as idiopathic, thus resembling our Case III (C. S.). From the evidence thus far, the relationship of arteritis to pulmonary hypertension is obscure but supports the view that pulmonary hypertension is a major factor in its pathogenesis.

The final point concerning our correlation of clinical and autopsy findings is related to the significance of thrombi in the pulmonary arterial circulation. That pulmonary hypertension develops following introduction of blood clot and other particulate material has been described by many investigators [*32,43*], and unrecognized emboli to the pulmonary arteries in man have been regarded as the basis for the development of pulmonary hypertension [*33*]. In two of our cases (Cases III and IV) canalized thrombi were noted. It is possible that the study of more material from the other two patients might have revealed similar lesions. If recurrent embolization is a common basis for pulmonary hypertension, then certain therapeutic implications are clear. However, the cases described and those studied subsequently strongly support the view that pulmonary hypertension can occur in the absence of pulmonary embolism. In a recent case, no evidence of embolism was observed postmortem in a patient with pulmonary hypertension, right ventricular failure, elevated venous pressure, congestive cirrhosis, ascites and edema of the lower extremities. No anatomic explanation for the pulmonary hypertension could be found but in the smaller branches of the pulmonary arteries the lesions formerly described, namely, plexiform lesions, intimal thickening and medial atrophy with fragmentation of the elastica and sinusoidal arteriolarcapillary communications, were found. These lesions as well as the concentric intimal thickening with atheromatosis of the larger branches we have interpreted as the effects of prolonged pulmonary hypertension. In another case, recent thrombosis was seen in association with a plexiform lesion, suggesting autochthonous thrombosis. It seems entirely likely that thrombi seen in various stages of organization in cases of pulmonary hypertension are autochthonous and do not originate as emboli. However, when pulmonary hypertension has been long-established and right ventricular failure has supervened, embolism can explain the presence of thrombi in the pulmonary arteries.

Whether primary or contributory, the role of thrombi appears important, and therefore the control or enhanced resorption of thrombi should be an aim in management.

SUMMARY

Primary pulmonary hypertension is a distinct clinicopathologic entity characterized by pulmonary arterial and right ventricular hypertension. Right ventricular hypertrophy is uniformly present and may or may not be accompanied by right ventricular failure. The hypertension is the result, hemodynamically, of excessive increase in pulmonary vascular resistance and may reflect itself in the pulmonary vessels by a variety of vascular lesions. The pathogenesis of the ultimate vascular lesions remains obscure. The clinical, hemodynamic, and pathologic features of four patients with this condition have been presented. The clinical findings of cases reported in the literature as well as in our cases show a remarkable uniformity which, in our interpretation, is a reflection of the pulmonary hypertension. Thus, it is difficult if not impossible on clinical grounds alone to differentiate this lesion from any of the multitude of conditions in which pulmonary hypertension is a secondary concomitant. Cardiac catheterization has provided valuable information on the pathophysiology of primary pulmonary hypertension and may be of great value in differential diagnosis, particularly in excluding congenital heart disease. The abnormal hemodynamic data that may be demonstrated by this technic, however, are not sufficiently specific to permit one to make a conclusive diagnosis. In our opinion, such a definitive diagnosis is possible only by necropsy, and even here the exact mechanism may be difficult to define, although it is apparent

now that many of the lesions in the pulmonary arteries represent the effect of pulmonary hypertension. In the face of such diagnostic difficulties and because there is no satisfactory treatment for primary pulmonary hypertension, it is in the patient's best interest that a search for a lesion that can be treated should not be abandoned.

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Effects of Aminophylline and Diamox Alone and Together on Respiration and Acid-Base Balance and on Respiratory Response to Carbon Dioxide in Pulmonary Emphysema*

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WHEN patients suffering from chronic pulmonary emphysema progress to the advanced stage in which they exhibit hypoxia and carbon dioxide retention, their chemical regulation of respiration becomes abnormal. Their respiratory centers are relatively insensitive to arterial pCO_2– (H^+) ion stimulation [*1*]. Respiration is sustained primarily by reflex hypoxic stimuli from the carotid and aortic body chemoreceptors [*2*]. This is reflected by respiratory depression following elevation of arterial pO_2 during inhalation of 100 per cent oxygen.

The concurrence of hypoxia and hypercarbia is not essential to the development of this altered regulation of respiration since a decreased sensitivity can develop in normal persons following sustained elevation of arterial pCO_2 by inhalation of 3 per cent CO_2 in air in the absence of hypoxia [*3*]. Furthermore, in the presence of a sustained decrease in the level of inspired pO_2, an increase in sensitivity to the arterial pCO_2– (H^+) ion stimulus develops in normal persons and they maintain a normal ventilatory response to an increase in arterial pO_2 [*4*]. These responses of normal subjects suggest that the dulling effect of a sustained elevation of arterial pCO_2 in patients with advanced emphysema contributes to their failure to adapt to a fall in the level of arterial pO_2.

In the early stage of chronic pulmonary emphysema, insufficiency of oxygen exchange (fall in arterial pO_2) precedes that of carbon dioxide exchange (rise in arterial pCO_2) [*5*]. In the moderately advanced stage the intensity and duration of hypoxia are apparently insufficient to bring about regulation of respiration by the chemoreceptors and when the level of arterial pCO_2, which is normal at rest, rises during exertion, it is probably not sustained for a sufficient length of time to dull the sensitivity of the respiratory centers.

The responsiveness of patients with advanced emphysema to the arterial pCO_2– (H^+) ion stimulus varies with the elevation of arterial pCO_2 and, to some extent, with plasma $BHCO_3^-$ [*6*]. Following the demonstration by Nadell [*7*] that diamox® can cause a sustained reduction to normal or nearly normal levels of arterial pCO_2 and plasma bicarbonate in such patients, interest was stimulated in the possibility that these biochemic changes might be associated with an increase in sensitivity of the respiratory centers and elimination of respiratory depression during inhalation of 100 per cent oxygen, that is, return of primary regulation of respiration to the respiratory centers. There is lack of agreement as to whether or not diamox tends to restore the respiratory sensitivity of these patients [*8*,*9*]. Our previously reported studies indicated that it does not eliminate respiratory depression during inhalation of 100 per cent oxygen [*10*]. †

† Studies in progress indicate that this is promptly overcome by the intravenous administration of 0.5 gm. aminophylline. It restores a normal pattern of respiration and stimulates respiration almost entirely by an increase in depth of breathing. This effect of a single intravenous

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TABLE 1

MEASUREMENTS OF SOME ASPECTS OF PULMONARY FUNCTION

Patient	Age (yr.)	Sex	Surface Area (M^2)	Total Lung Capacity		Ratio of Residual Volume to Total Lung Capacity (%)	Vital Capacity*					Maximum Breathing Capacity*				
							Control		Aminophylline (% of predicted)	Diamox (% of predicted)	Diamox and Aminophylline (% of predicted)	Control		Aminophylline (% of predicted)	Diamox (% of predicted)	Diamox and Aminophylline (% of predicted)
				(ml.)	(% of predicted)		(ml.)	(% of predicted)				(L.)	(% of predicted)			
Patients with Moderate Emphysema																
R. A.	50	M	1.90	4807	83	35	3315	83	87	..	..	102	100	100	..	..
J. P.	62	M	1.46	4015	88	41	2345	75	80	72	79	32	45	48	42	48
F. N.	59	M	1.82	5096	98	54	2043	57	65	..	..	51	57	64	..	..
Patients with Advanced Emphysema																
M. A.	64	M	1.69	5326	104	57	2528	72	81	76	96	25	30	38	40	43
G. R.	60	M	1.38	3710	76	65	1601	48	60	55	54	20	29	29	27	29

* The average of the best of three measurements carried out while standing at the end of several observations in each period. All measurements of lung volumes and maximum breathing capacity corrected to BTPS (body temperature and pressure, saturated with water vapor).

The present studies are primarily concerned with determining whether or not aminophylline and diamox separately increase the diminished sensitivity of the respiratory centers in patients with advanced emphysema, and whether or not sustained reduction in the level of arterial pCO_2 and plasma $BHCO_3^-$ induced by diamox enhances the response to aminophylline. If the responsiveness of the respiratory centers of such patients was increased by the administration of these drugs alone or together, it would be of considerable theoretic and therapeutic interest. Aminophylline was selected not only for its effect in stimulating respiration, but also because its bronchodilator action would, in the presence of bronchoconstriction, tend to decrease the work of breathing. The respiratory effects of 3.3 to 4.3 mg./kg. diamox administered every twelve hours in this study were essentially the same as those previously reported in which a comparable dose was administered every six hours [*10*] and therefore will not be discussed in detail. These findings agree in many respects with those of other workers [*11*].

dose (6 mg./kg.) may last one and a half to two hours while the patient continues to breathe oxygen. When this dose is promptly followed by a constant infusion of 100 mg. of aminophylline per hour in 5 per cent glucose in water which is continued for as long as four hours, respiratory stimulation is sustained and a significant rise in arterial pCO_2 does not occur while the patient breathes oxygen. Five per cent glucose in water administered in place of aminophylline has been without beneficial effect.

METHODS

The five patients studied were well trained in the experimental procedures. Three (R. A., J. P. and F. N.) had moderately advanced and two (M. A. and G. R.) had advanced pulmonary emphysema. (Table 1). All patients had been observed for a period of several months to several years.

Observations were made in each study under basal conditions with the head of the bed raised to the same reclining angle. An indwelling arterial needle was inserted into the lumen of the right brachial artery under 2 per cent procaine hydrochloride local anesthesia. Air was inspired through one arm of a two-way valve and expired through another into a Tissot spirometer of 100 L. capacity. The dead space of the valve and mouthpiece was 55 ml. Tidal volume, respiratory rate and minute volume were recorded on a constant speed ink-recording drum attached to the spirometer. Several two-minute periods were recorded to insure constancy of the respiratory minute volume and filling of the dead space of the Tissot spirometer with expired air. Arterial blood was collected anaerobically, using heparin solution as an anticoagulant, during the middle two and one-half minutes of a four-minute period of expired air collection.

In the aminophylline studies 0.5 gm. in 20 cc. water was administered intravenously over a period of five to seven minutes following stabilization of respiratory minute volume and simultaneous collection of expired air and arterial blood samples. In the preliminary studies minute volume of respiration was recorded during two minutes of every four minutes over a period of approximately forty minutes following aminophylline injection. Arterial blood and expired air samples were then collected as in the control

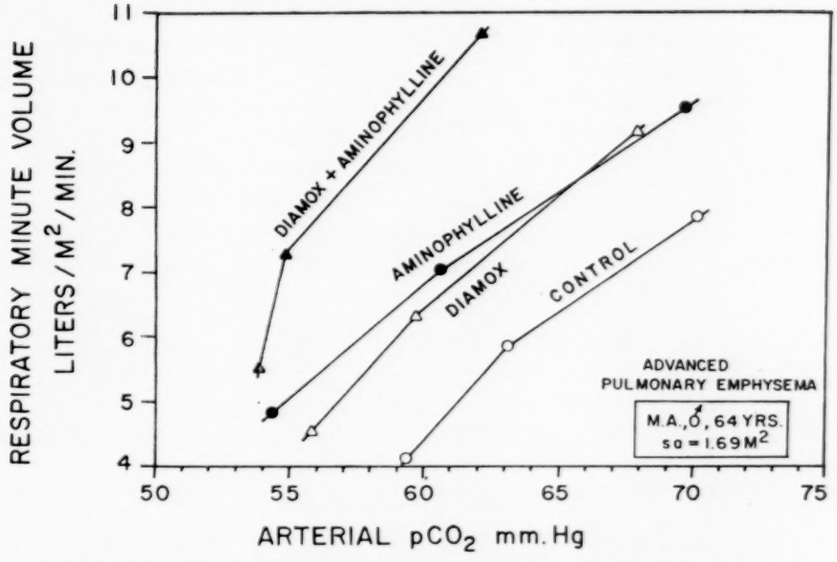

FIG. 1. Effect of aminophylline and diamox, alone and together, on the respiratory response to increased arterial pCO_2. Curves drawn through means of values. The lowest point in each curve represents observations during inhalation of 100 per cent oxygen, the next during inhalation of a mixture of 2.54 per cent CO_2 and 93.46 per cent oxygen and the highest point during inhalation of a mixture of 4.91 per cent CO_2 and 95.09 per cent oxygen.

period. Later, the protocol was changed so that minute volume was measured and samples of expired air collected during injection of aminophylline and the following forty minutes in four- to six-minute periods with intervening two minutes for withdrawal of duplicate samples of expired air for analysis and expulsion of the remaining expired air from the spirometer. Arterial blood and expired air were then collected as previously described. Arterial blood pressure was measured in the left arm with a mercury manometer and the pulse rate was recorded at frequent intervals throughout each study. Oral temperature was also recorded at the start, approximately at the mid-point and shortly after the end of each study. In no instance was there an abnormal rise in oral temperature.

The diamox studies were begun after the drug had been administered every twelve hours for at least five days to be sure that its inhibitory effect on renal tubular $BHCO_3^-$ reabsorption had stabilized. Arterial blood and expired air samples were collected two to three hours after a morning dose of diamox. The pH of urine specimens voided under oil at the end of some of the studies was always acid with nitrazine paper. The same protocol was followed when aminophylline was administered while the patients were receiving diamox as during the control period and when 20 cc. of 0.85 normal saline solution were injected in place of aminophylline.

The studies with the mixtures of approximately 3 and 5 per cent CO_2 in O_2 were begun about fifteen minutes after completion of control and aminophylline studies. Each gas mixture was delivered from a large commercial tank to an airtight humidifier and then by way of an anesthesia rubber bag with a capacity of 15 L., and breathed for thirty minutes. Minute volume of respiration was recorded continuously except for the time necessary to express the expired air from the Tissot spirometer. Arterial blood was drawn during the middle two and one-half minutes of the last four minutes of collection of expired air. About twenty minutes of breathing room air elapsed before the study with the mixture of the higher concentration of CO_2 in oxygen was begun. In some instances studies were also carried out with 100 per cent oxygen in place of the CO_2-O_2 mixtures. Measurements of vital capacity and maximum breathing capacity were begun about fifteen to twenty minutes following the gas mixture studies and completed about two and one-half to three hours following injection of aminophylline or saline.

Curves were drawn through the points which define minute ventilation and level of arterial pCO_2 in the 100 per cent oxygen and the two CO_2-O_2 gas mixtures for each study. In patients with advanced emphysema, ventilation was often depressed and quite irregular during inhalation of 100 per cent oxygen in the control and diamox periods, and the curves joining all three points did not always approximate a straight line. However, the portion of the curves joining the mean of the observations with the CO_2-O_2 gas mixtures in each period were more or less parallel to one another. (Fig. 1.) Two criteria were employed to evaluate the effect of the drugs

under investigation. One is the slope of response, that is, rate of increase in minute ventilation per mm. Hg rise in arterial pCO_2 during inhalation of the CO_2-O_2 gas mixtures.* This is considered an index of sensitivity or irritability of the respiratory centers. The other is the minute ventilation at an arbitrarily selected level of arterial pCO_2 through which the response curves of each patient pass in the different study periods. According to Loeschke et al. this is the most sensitive index of respiratory stimulation or depression and it has the advantage of eliminating differences in arterial pCO_2 attained during inhalation of the same CO_2-O_2 mixtures [*12*]. When a response curve is displaced parallel to a control period curve the sensitivity of the respiratory centers is obviously unchanged; however, its threshold is believed altered. The threshold is decreased when the response curve is displaced upward and parallel to the control curve and it is lowered when it is displaced in the opposite direction.

Arterial blood oxygen, carbon dioxide contents [*13*] and arterial blood hemoglobin oxygen capacity [*14*] were analyzed in a Van Slyke manometric apparatus. Arterial pH was measured with a sealed glass microelectrode immersed in a 37°c. water bath, in a Model G Beckman pH meter. Arterial pCO_2 and plasma bicarbonate were calculated by the Henderson-Hasselbalch equation. Oxygen and carbon dioxide contents of inspired and expired air were measured in a micro-Scholander apparatus [*15*].

Residual air volume was measured by the open circuit method of Darling, Cournand and Richards [*16*] with modifications as previously published [*17*]. This, together with vital capacity, is a measure of the total lung volume. Vital capacity and maximum breathing capacity were measured in a Collins ventilometer. They represent the maximum volumes attained in a series of three observations at one sitting in a day.

RESULTS

Immediate Effects of 0.5 gm. (6.9 to 10.9 mg./kg.) Aminophylline and of 0.85 Normal Saline Solution Administered Intravenously during Control and Diamox Periods. There was some variation in the sequence and intensity of changes in minute ventilation, oxygen uptake, respiratory gas exchange ratio (R.Q.), arterial blood pCO_2 and acid base balance during the five to seven minutes of injection of aminophylline and the following forty minutes. An example is illustrated in Table II. During the period of injection minute ventilation generally increased promptly, as much as 10 to 25 per cent. Peak increase in ventilatory stimulation generally occurred during the ten to twenty minutes following completion of the injection. Over the following several minutes it tended to return to control level. During the last period of observation moderate ventilatory stimulation without significant increase in respiratory gas exchange ratio (R.Q.) was generally present, alveolar pO_2 and arterial blood oxygen saturation were sometimes increased, arterial pCO_2 was either at or a few mm. Hg below control level, and arterial pH reflected the trend in arterial pCO_2, since plasma $BHCO_3^-$ level did not change. (Tables III and IV.)

When aminophylline was administered concurrently with diamox the changes were generally in the same direction, and somewhat more marked. Control studies with 20 cc. of 0.85 normal saline solution administered intravenously in place of aminophylline were unassociated with significant alterations in the ventilatory and blood measurements.

The significance of the difference between mean control observations and those approximately three-fourths of an hour following administration of aminophylline alone and together with diamox, and two to three hours following a morning dose of diamox, are presented in Tables III and IV. Aminophylline caused a rise in minute ventilation in four of the five patients (all but R. A.); in no instance was there an alteration in respiratory gas exchange ratio (R.Q.). Arterial pCO_2 fell in two patients (F. N. and M. A.); in one of whom (M. A.) this was sufficient to raise arterial pH from 7.34 to 7.39. There were insignificant fluctuations in the level of plasma bicarbonate. A rise in oxygen uptake usually accompanied the rise in minute ventilation.

When aminophylline was administered two to three hours after a morning dose of diamox, all except patient J. P. exhibited greater ventilatory stimulation than when each drug was studied separately. (Table III.) This ventilatory stimulation was not associated with a further increase in oxygen uptake. Arterial pCO_2 fell 4 to 6 mm. Hg below the mean of the diamox period in three patients (R. A., J. P. and G. R.) in whom it was sufficient to compensate partially or completely the diamox-induced metabolic acidosis. In all five patients the absolute fall in the level of arterial pCO_2 was highly significant as compared with control levels. Alveolar pO_2 level rose 5 to 6 mm. Hg

* Other workers have also employed two points to measure the response of normal subjects [*12*] and patients with emphysema [*6*] to the arterial pCO_2– (H^+) ion stimulus.

TABLE II

EXAMPLE OF IMMEDIATE RESPONSE TO 0.5 GM. INTRAVENOUS AMINOPHYLLINE (6.9 MG./KG.) IN PATIENT R. A. WITH MODERATE EMPHYSEMA

Minutes	$\dot{V}_E/M^2$ (L./min.)	$\dot{V}_{O_2}/M^2$ (ml./min.)	R	Arterial Blood				$P_{A_{O_2}}$ (mm. Hg)
				$P_{a_{CO_2}}$ (mm. Hg)	Plasma $BHCO_3^-$ (mEq./L.)	pHa	Sa_{O_2} (%)	
Control Period								
−29 −25	4.39	...	...	....	....	....	....	...
−23 −19	4.25	...	...	....	....	....	....	...
−17 −14	4.27	129	.76	37.6	25.4	7.45	91.2	103
Aminophylline Period								
0– 6*	5.14	159	.81	....	....	....	....	...
9–15	6.96	187	.85	....	....	....	....	...
17–22	4.89	136	.80	....	....	....	....	...
24–30	5.00	149	.78	....	....	....	....	...
32–38	4.52	148	.72	....	....	....	....	...
40–44	4.58	142	.72	34.7	24.5	7.47	97.3	105

NOTE: $\dot{V}_E/M^2$ = minute volume of expired air, BTPS (body temperature and pressure, saturated with water vapor) per square meters of body surface.

$\dot{V}_{O_2}/M^2$ = oxygen consumption, STPD (standard temperature and pressure, dry) per square meters of body surface.

R = CO_2-O_2 respiratory exchange ratio (R.Q.) expired air.

$P_{a_{CO_2}}$ = carbon dioxide tension, arterial blood.

$BHCO_3^-$ = bicarbonate, mEq./L.

pHa = arterial blood pH.

Sa_{O_2} = oxygen saturation, arterial.

PA_{O_2} = effective alveolar oxygen tension.

* During aminophylline injection.

above the mean of the diamox period in three patients and the absolute increase above control levels was significant in all patients. (Table IV.)

Respiratory and Metabolic Effects of Prolonged Administration of 3.3 to 4.3 mg./kg. Diamox Every Twelve Hours. The mean values of observations carried out two to three hours after a morning dose of diamox over one to thirty-four weeks are summarized in Tables III and IV. Arterial pCO_2 level fell 5 to 9 mm. Hg in four of the five patients, the same as or 2 to 4 mm. Hg more than following treatment with aminophylline. The remaining patient, J. P., exhibited no change either with administration of diamox or aminophylline. The plasma bicarbonate level fell 4.3 to 7.8 mEq./L. in all five patients. When the level of arterial pH fell in three of the five patients (J. P., F. N. and M. A.) it reflected the relatively greater reduction in plasma $BHCO_3^-$ than arterial pCO_2. Alveolar pO_2 rose in four of the five patients and in two (F. N. and G. R.) it was 4 to 6 mm. Hg higher than it was following dosage of aminophylline.

Effects of Aminophylline, Diamox and Both Together on Respiratory Pattern, Vital Capacity and Maximum Breathing Capacity. When ventilatory stimulation occurred it was, in nearly all instances, associated with an increase in depth of breathing while the rate generally remained the same. (Table IV.) A moderate increase in vital capacity with administration of aminophylline alone and together with diamox, and in maximum breathing capacity in all three drug periods occurred in patient M. A. (Table I.) Patient G. R. exhibited only an increase in vital capacity following administration of aminophylline. None of the other patients exhibited a change in vital capacity or maximum breathing capacity. The other ventilatory measurements, total lung capacity and ratio of residual to total lung capac-

TABLE III

MEAN VALUES OF SOME EFFECTS OF AMINOPHYLLINE, DIAMOX AND BOTH TOGETHER ON RESPIRATION, ACID-BASE BALANCE AND ARTERIAL BLOOD OXYGEN SATURATION

Patient	Dose		Respiration												Arterial Blood												Plasma			
			$\dot{V}_E/M^2$ (L./min.)				R				$\dot{V}_{O_2}/M^2$ (ml./min.)				pHa				$PaCO_2$ (mm. Hg)				Sa_{O_2} (%)				$BHCO_3^-$ (mEq./L.)			
	Aminophylline (mg./kg.)	Diamox (mg./kg.)	Control	Aminophylline	Diamox	Diamox and Aminophylline	Control	Aminophylline	Diamox	Diamox and Aminophylline	Control	Aminophylline	Diamox	Diamox and Aminophylline	Control	Aminophylline	Diamox	Diamox and Aminophylline	Control	Aminophylline	Diamox	Diamox and Aminophylline	Control	Aminophylline	Diamox	Diamox and Aminophylline	Control	Aminophylline	Diamox	Diamox and Aminophylline
Patients with Moderate Emphysema																														
R. A.	6.9	4.2	(7)* 4.25	(4) 4.30	(3) 4.30	(3) 5.15†	.82	.82	.80	.79	119	116	118	114	7.36	7.41	7.30	7.34	43.9	39.3	35.6‡	30.1†	91.0	95.1	94.8	97.8	23.9	24.0	16.8†	15.8†
J. P.	9.6	2.9	(6) 8.0	(5) 9.88†	(7) 7.52	(3) 9.18†	.82	.82	.81	.77	155	171	133‡	153	7.40	7.41	7.32†	7.38	39.4	37.2	38.2	32.2†	91.6	94.3	93.3	96.3	23.7	23.0	19.1†	18.3†
F. N.	7.2	7.2	(6) 5.59	(3) 6.62†	(2) 6.45†	(2) 8.14†	.81	.77	.86‡	.87	115	128†	114	120	7.40	7.44	7.33†	7.34†	40.2	35.3†	33.2†	30.8†	88.4	89.8	87.7	89.7	24.3	23.4	16.5†	16.0†
Patients with Advanced Emphysema																														
M. A.	8.7	4.3	(12) 4.35	(7) 4.86†	(3) 4.84†	(3) 6.08†	.79	.76	.81	.80	121	133‡	119	137‡	7.34	7.39†	7.28‡	7.29‡	56.9	48.2†	48.3†	45.0†	....	....	....	....	29.5	28.0	22.1†	21.0†
G. R.	10.9	3.3	(5) 5.06	(3) 5.69†	(10) 5.31	(6) 6.07†	.75	.74	.79‡	.75	116	128	111	121	7.38	7.43	7.34	7.39	48.5	44.5	43.9‡	40.4†	88.3	92.5	91.7	96.1	27.7	28.5	23.4†	23.6†

NOTE: $\dot{V}_E/M^2$ = minute volume of expired air, BTPS per square meters of body surface area.
R = CO_2-O_2 respiratory exchange ratio (R.Q.) expired air.
$\dot{V}_{O_2}/M^2$ = oxygen consumption, STPD (Standard temperature and pressure, dry) per square meters of body surface.
pHa = arterial blood pH.
$PaCO_2$ = carbon dioxide tension, arterial blood, mm. Hg.
Sa_{O_2} = oxygen saturation, arterial blood, per cent.
$BHCO_3^-$ = bicarbonate, mEq./L.

* The numbers in parentheses indicate the number of observations in each period and apply to all the measurements listed except for oxygen saturation of arterial blood which is the average generally of two to five observations and insufficient for statistical evaluation. Observations were made forty-five minutes after the start of injection of aminophylline and two to three hours after a morning dose of diamox.
† P = <0.01.
‡ P = <0.05.

TABLE IV

MEAN VALUES OF EFFECT OF AMINOPHYLLINE AND DIAMOX ALONE AND TOGETHER ON DEPTH AND FREQUENCY OF RESPIRATION AND ALVEOLAR PO_2 MM. HG

Patient	Dose mg./kg.		V_T (ml.)				f (breath/min.)				P_{AO_2} (mm. Hg)			
	Aminophylline	Diamox	Control	Aminophylline	Diamox	Diamox and Aminophylline	Control	Aminophylline	Diamox	Diamox and Aminophylline	Control	Aminophylline	Diamox	Diamox and Aminophylline
Patients with Moderate Emphysema														
R. A.	6.9	4.2	495	470	534	581*	16.5	17.6	15.5	17.1	97	104	106†	112*
J. P.	9.6	2.9	451	501	458	521	26.0	29.2	24.0*	25.8	103	105	104	109†
F. N.	7.2	7.2	500	521	609*	690*	20.5	23.2*	19.4	21.3	(5) 100	106†	110	113*
Patients with Advanced Emphysema														
M. A.	8.7	4.3	418	463*	501*	611*	17.8	17.9	16.9	17.3	80	(6) 89*	90†	95*
G. R.	10.9	3.3	324	344*	362*	398*	22.0	23.4*	20.7	21.4	86	90	96*	98*

NOTE: The dose, number and sequence of observations correspond to those listed in Table III except as indicated in patients R. A. and F. N. in whom fewer measurements of alveolar pO_2 were made.

V_T = Tidal volume, BTPS. f = Respiratory frequency. P_{AO_2} = Effective alveolar oxygen tension.

* $p = <0.01$.

† $p = <0.05$.

ity, carried out in the control period are listed in Table I.

Effect of Aminophylline, Diamox and Both Together on the Ventilatory Response to 100 Per Cent O_2 and Mixtures of Approximately 3 and 5 Per Cent CO_2 in O_2. Minute ventilation was often depressed and periodic in patients with advanced emphysema while breathing 100 per cent oxygen and was stable during the last five to ten minutes of the two CO_2-O_2 gas mixture periods. The segments of the curves drawn through the points which define the mean values of arterial pCO_2 and minute ventilation in the CO_2-O_2 mixture periods for each patient were displaced in a more or less parallel manner from one another in the different study periods, indicating that there was a change in threshold and not in sensitivity of response. (Fig. 1.) Tables V and VI summarize observations during inhalation of the CO_2-O_2 gas mixtures. Only patients with moderate emphysema (R. A. and J. P.) exhibited a normal sensitivity of response in the control period and in none did it increase in the drug periods. (Table VI.) The threshold fell in each of the drug periods in one patient (R. A.) with moderate emphysema; following administration of aminophylline, in a patient (M. A.) with advanced emphysema; and after treatment with aminophylline together with diamox, in both patients with advanced emphysema, as reflected by the increase in minute ventilation at an arbitrarily selected level of arterial pCO_2. (Table VI.) In the latter two patients the increase in response following the concurrent administration of aminophylline and diamox exceeded the sum of the separate effects of each drug. (Table VI.) Patient J. P. exhibited a decrease in slope and little or no alteration in intensity of response at 46 mm. Hg arterial pCO_2 in the different drug periods. (Table VI.) This was likely due to a marked increase in the work of breathing since he experienced shortness of breath while breathing the 4.6 per cent CO_2 in oxygen mixture in these periods.

COMMENTS

The effectiveness of aminophylline as a respiratory stimulant, other than in the presence of Cheyne-Stokes respiration [*18*], has been disputed [*19–21*]. Differences in dose and route of administration employed may account for this. The painful effect of intramuscular injection of aminophylline may have contributed to the respiratory stimulation reported by Starr and his associates [*19*] following a 0.48 gm. dose in normal subjects. The slow and irregular rate of absorption of a relatively small subcutaneous 0.25 gm. dose may explain the failure of Richmond [*20*] to note respiratory stimulation in young healthy adults. Studies of the effects of

TABLE V

EFFECT OF AMINOPHYLLINE AND DIAMOX ALONE AND TOGETHER ON RESPIRATORY MINUTE VOLUME AND ACID BASE BALANCE DURING INHALATION OF CO_2-O_2 MIXTURES*

Patient	Period	No. Observations	$\dot{V}_E/M^2$ (L./min.)	$PaCO_2$ (mm. Hg)	pHa	Plasma $BHCO_3^-$ (mEq./L.)	$\dot{V}_E/M_2$ (L./min.)	$PaCO_2$ (mm. Hg)	pHa	Plasma $BHCO_3^-$ (mEq./L.)
Patients with Moderate Emphysema										
			2.73% CO_2				4.91% CO_2			
R. A.	Control	3	6.67 ± .37	49.1 ± 2.8	7.32 ± .05	24.8 ± 1.5	11.95 ± .79	55.1 ± 2.9	7.20 ± .40	25.1 ± 1.2
	Aminophylline	2	9.12 ± .64	41.0 ± 1.3	7.39 ± .04	23.8 ± 1.2	17.05 ± 2.9	48.3 ± 3.6	7.34 ± .02	25.0 ± 0.6
	Diamox	2	9.48 ± .94	36.7 ± 1.0	7.28 ± .01	16.8 ± 1.1	22.45 ± 1.32	48.8 ± .28	7.19 ± .01	17.8 ± 0.4
	Diamox and aminophylline	2	13.56 ± .87	32.5 ± 2.6	7.32 ± .05	16.0 ± 0.6	25.29 ± .09	46.1 ± 1.7	7.20 ± .02	17.3 ± 0.2
			2.73% CO_2				4.30% CO_2			
J. P.	Control	3	10.49 ± 1.91	47.6 ± 6.2	7.34 ± .06	25.0 ± 2.1	12.66 ± 1.2	50.8 ± 5.4	7.33 ± .06	25.9 ± 1.3
	Aminophylline	3	13.12 ± .18	41.9 ± 3.3	7.39 ± .04	24.4 ± 1.3	15.74 ± .60	48.5 ± 3.7	7.33 ± .06	25.3 ± 1.4
	Diamox	3	10.91 ± .36	43.2 ± 1.4	7.29 ± .02	19.9 ± 0.9	13.01 ± .42	52.6 ± 2.3	7.21 ± .02	20.2 ± 1.0
	Diamox and aminophylline	3	13.39 ± .90	41.8 ± 1.4	7.30 ± .02	19.9 ± 0.3	16.41 ± . 58	52.4 ± 1.8	7.21 ± .01	20.4 ± 0.6
Patients with Advanced Emphysema										
			2.54% CO_2				4.91% CO_2			
M. A.	Control	3	5.86 ± .31	63.1 ± 3.4	7.28 ± .04	28.8 ± 0.6	7.89 ± .39	70.1 ± 1.9	7.25 ± .02	29.6 ± 0.2
	Aminophylline	3	7.07 ± .63	60.6 ± 3.6	7.28 ± .03	27.8 ± 1.4	9.46 ± .70	69.6 ± 4.2	7.23 ± .05	28.2 ± 1.8
	Diamox	4	6.26 ± .11	59.7 ± 3.4	7.22 ± .04	23.7 ± .70	9.16 ± .41	67.8 ± 2.3	7.19 ± .06	24.3 ± 1.0
	Diamox and aminophylline	3	7.28 ± .16	54.8 ± 2.4	7.26 ± .03	24.4 ± .64	10.62 ± 1.04	62.1 ± 2.4	7.23 ± .02	25.1 ± 0.62
			2.54% CO_2				4.30% CO_2			
G. R.	Control	3	5.95 ± .45	58.8 ± 2.7	7.29 ± .03	27.6 ± 0.3	7.31 ± .45	64.6 ± 1.2	7.26 ± .03	28.1 ± 0.8
	Aminophylline	2	7.27 ± .22	59.8 ± 2.3	7.30 ± .01	28.3 ± 0.6	8.94 ± .68	64.6 ± 3.4	7.26 ± .01	28.1 ± 0.5
	Diamox	4	7.17 ± .26	55.8 ± 2.1	7.24 ± .04	22.8 ± 0.2	8.87 ± .43	60.5 ± 1.7	7.21 ± .04	23.4 ± 0.8
	Diamox and aminophylline	4	8.02 ± .16	50.1 ± 2.1	7.29 ± .04	23.0 ± 1.2	10.20 ± .51	58.4 ± 2.6	7.23 ± .04	23.5 ± 0.9

NOTE: $\dot{V}_E/M^2$ = minute volume of expired air, BTPS per square meters of body surface.
$PaCO_2$ = carbon dioxide tension, arterial blood.
pHa = arterial blood pH.
$BHCO_3^-$ = bicarbonate.
* Mean values and standard deviation.

intravenously administered 0.5 gm. aminophylline on cerebral circulation and oxygen consumption suggested that it stimulates respiration, since it was associated with a fall in the level of arterial pCO_2 [22].

In the preliminary phase of the studies it was observed that intravenous administration of 0.5 gm. aminophylline was associated with a prompt increase in minute ventilation and, in many instances, with tachycardia and complaints of nervousness, mild "sickness to the stomach," tightness about the head and perioral tingling. The subjective reactions generally subsided within ten to fifteen minutes. Minute ventilation gradually fell, but remained slightly above the control level and a moderate tachycardia frequently persisted during the thirty to forty minutes of observation following injection. These observations indicated the need to clarify whether the prompt increase in minute ventilation reflects an increase in metabolic rate or is due to respiratory stimulation. It also appeared essential to evaluate the bronchodilator action of aminophylline in a study of its respiratory effects in patients with emphysema.

Immediate Effects of Aminophylline Alone and To-

TABLE VI

STATISTICAL EVALUATION OF EFFECT OF AMINOPHYLLINE AND DIAMOX ALONE AND TOGETHER ON SENSITIVITY OF RESPIRATORY RESPONSE TO ARTERIAL PCO_2 AND $(H^+)\alpha$ AND ON RESPIRATORY MINUTE VOLUME AT A FIXED LEVEL OF ARTERIAL PCO_2 BASED ON CURVES FOR EACH STUDY WITH CO_2-O_2 GAS MIXTURES

Patient	Period	No. Observations	$\frac{\dot{V}_E/M^2}{PaCO_2}$ (L./min.)	$\frac{\dot{V}_E/M^2}{(H^+)a}$ (L./min.)	$\dot{V}_E/M^2$ at Fixed $PaCO_2$		
					$PaCO_2$ (mm. Hg)	$\dot{V}_E$ (L./min.)	Above Control (%)
Patients with Moderate Emphysema							
R. A.	Control	3	.94 ± .27	1.74 ± 1.51		5.5 ± 2.3	
	Aminophylline	2	1.20 ± .33	3.24 ± 2.94		17.4* ± 1.4	216
	Diamox	2	1.07 ± .03	1.01 ± 0.13	48	21.6* ± 1.6	293
	Diamox and aminophylline	2	.86 ± .00	.77 ± 0.71		26.9* ± 1.4	389
J. P.	Control	3	.87 ± .36	1.60 ± .18		11.6 ± 0.7	
	Aminophylline	3	.51 ± .35	1.16 ± 1.42		15.1† ± 1.3	31
	Diamox	3	.22 ± .01	.20 ± .03	46	11.6 ± 0.8	0
	Diamox and aminophylline	3	.27 ± .08	.27 ± .09		14.6† ± 0.5	27
Patients with Advanced Emphysema							
M. A.	Control	3	.29 ± .03	.46 ± .09		5.7 ± .7	
	Aminophylline	3	.29 ± .64	.48 ± .37		7.4† ± .2	30
	Diamox	4	.50 ± .34	.97 ± 1.00	62	6.8 ± 1.4	19
	Diamox and aminophylline	3	.57 ± .38	1.27 ± 1.45		10.8* ± 1.6	90
G. R.	Control	3	.23 ± .02	.36 ± .12		5.8 ± 1.0	
	Aminophylline	2	.36 ± .08	.40 ± .09		6.7 ± 0.1	16
	Diamox	4	.37 ± .08	.69 ± .58	58	8.0 ± 1.4	38
	Diamox and aminophylline	4	.33 ± .20	.35 ± .20		10.2* ± 1.3	78

NOTE: $\frac{\dot{V}_E/M^2}{PaCO_2}$ = increase in minute volume of expired air, BTPS per square meter body surface area associated with a rise of 1 mm. Hg in arterial CO_2 tension.

$\frac{\dot{V}_E/M^2}{(H^+)a}$ = increase in minute volume of expired air, BTPS per square meter body surface area associated with a rise in arterial hydrogen ion concentration of 1 billionth of a mole per liter.

$PaCO_2$ = carbon dioxide tension, arterial blood.

$\dot{V}_E/M^2$ = minute volume of expired air, BTPS per square meters of body surface area.

$\dot{V}_E$ = minute volume of expired air, BTPS.

*p = <0.01.

†p = <0.05.

gether with Diamox on Ventilation and Oxygen Uptake. Increase in minute ventilation and somewhat greater increase in oxygen uptake generally occurred during the intravenous administration of 0.5 gm. aminophylline during control and diamox periods. These effects were usually well sustained over the following ten to twenty minutes when ventilatory stimulation often exceeded the increase in oxygen uptake. Thereafter, both tended to return to control level. However, during the last period of observation, moderate ventilatory stimulation without increase in oxy-

gen uptake was generally present. (Table III.) The fact that this was often associated with a fall in arterial pCO_2 and a rise in arterial blood hemoglobin oxygen saturation indicates that more effective integration of alveolar ventilation and circulation occurred. The increase in arterial blood oxygen content, per cent hemoglobin saturation and of tissues with oxygen which followed the administration of aminophylline in these anoxemic patients, probably accounted for the major portion of the increase in rate of oxygen uptake. (Tables II and III.)

EVALUATION OF RESPIRATORY STIMULANTS

While Breathing Room Air. The evidence cited herein for a respiratory stimulating action of aminophylline alone and together with diamox is not impressive. It reflects the limitations of attempts to evaluate the respiratory effects of drugs while the subject breathes room air [*12*]. A normally responsive respiratory regulatory mechanism, as exhibited by (R. A. and J. P.) two patients with moderate emphysema (Tables V and VI), is sensitive to minor changes in the level of arterial pCO_2 and/or (H^+) ion concentration and tends to maintain these stimuli within a narrow range by prompt adjustments in respiratory minute volume. Thus the action of respiratory stimulants is considerably limited by their effect on the level of arterial pCO_2 and/or (H^+) ion concentration.

The fact that the respiratory regulatory mechanism of patients with advanced emphysema, such as G. R. and M. A., is relatively unresponsive to changes in the level of arterial pCO_2 and/or (H^+) ion concentration (Tables V and VI) and is sustained primarily by hypoxic stimuli arising in the carotid and aortic chemoreceptors, presents an additional limitation to the evaluation of the respiratory effects of drugs while subjects breathe room air. Hyperventilation induced by a respiratory stimulant tends to raise the abnormally low alveolar pO_2. This may be sufficient to afford considerable relief from hypoxia. The response to the respiratory stimulant then may reflect a compromise between the depressant effect of partial relief of hypoxia and the stimulant effect of the drug.

While Breathing CO_2-O_2 Mixtures. Mixtures of low concentrations of CO_2 in O_2 rather than in room air are preferred by some investigators in order to attain consistent and maximum relief from hypoxia in studies of the ventilatory response of patients with emphysema to increased concentrations of CO_2 [*6,8*]. This method is of limited value and relatively insensitive when the minute volume of respiration is related to the inspired pCO_2 mm. Hg or volume per cent (as carried out by some, [*6,9*]) rather than to the level of arterial pCO_2 and/or (H^+) ion concentration attained, since this is the respiratory stimulus. Furthermore, the arterial pCO_2 level during inhalation of the same CO_2 gas mixture may vary in the same subject in different studies and is not the same in all subjects. Since we could not predict how varying degrees of relief of hypoxia might influence the ventilatory response to increased levels of arterial pCO_2 and/or (H^+) ion concentration, 100 per cent oxygen and mixtures of approximately 3 and 5 per cent CO_2 in O_2 were employed.

Effect of Aminophylline, Diamox and Both Together on the Response of the Respiratory Regulatory Mechanism to the Arterial pCO_2– (H^+) Ion Stimulus. In the control period the responsiveness of patients with moderate emphysema was normal and of the patients with advanced emphysema, considerably reduced (Table VI) according to the observations of Prime and Westlake [*6*]. These patterns of response were unaltered even in the presence of a reduction in the level of arterial pCO_2 with aminophylline, and of arterial pCO_2 and plasma $BHCO_3^-$ with diamox alone and together with aminophylline. This was to some extent unexpected in patients R. A. and J. P., since in the presence of normally responsive respiratory centers which they exhibited, the sensitivity increases following sustained decrease in the level of arterial pCO_2 and plasma $BHCO_3^-$ as a result of altitude adaptation [*4,23*] or mechanical hyperventilation in a respirator [*24*].

The consensus is that depression of the sensitivity of the respiratory centers of patients with advanced emphysema reflects cellular adaptation to prolonged elevation of arterial pCO_2 [*8*]. The fact that this correlates fairly well with the degree of retention of arterial pCO_2 [*1,8*] and CO_2 content [*25,26*] stimulated us and others [*8,9*] to study whether or not the sensitivity of the respiratory centers of patients with advanced emphysema could be enhanced by reduction in these levels toward normal following the administration of diamox.

Fishman and his co-workers [*9*] were unable to demonstrate such a relationship in a study of the effects of varied concentrations of CO_2 in inspired air mixtures, following restoration and

maintenance over several months of a normal level of arterial pCO_2 and plasma $BHCO_3^-$ by the ingestion of diamox. They concluded that once established the diminished sensitivity of such patients to CO_2 is irreversible. The observations reported herein, judged by the level of arterial pCO_2 rather than inspired pCO_2, indicate that diamox does not alter responsiveness either in the presence of a normal sensitivity (patient R. A.), or of a diminished sensitivity (patients G. R. and M. A.). (Table VI.)

The fact that patient J. P. exhibited normal responsiveness during the control period and an apparent decrease during the drug periods (Table VI), indicates that disturbances in the mechanics of respiration may be sufficient to limit the response. This no doubt reflects the shortness of breath and fatigue (and probable marked increase in work of breathing) which he, of all the patients studied, experienced while inhaling the higher concentration of CO_2 in O_2 mixture in the drug periods. Others [*6,8*] have cited a few such similar instances, and plots of the stimulus response curves, like those of patient J. P., also tended to become concave downward [*8*]. Although this may be important in some patients, it did not appear to contribute to the limited response exhibited by patients with advanced emphysema in the present study and also in those studied by Alexander [*1*], Fishman [*9*] and their co-workers. Similarly, mechanical ventilatory disability does not account for the failure of the sensitivity of patient R. A. to increase, since he had a normal maximum breathing capacity. (Table I.) Relief of bronchoconstriction by a nebulized bronchodilator (isuprel®) in patients with emphysema decreases the work of breathing caused by viscous and elastic resistance to respiration [*27*]. When aminophylline has a potent bronchodilator action, it too would be expected to have a similar effect. Were this its only action, it should have brought about a decrease in minute volume of respiration and in oxygen uptake and an apparent increase in sensitivity of response to the arterial pCO_2– (H^+) ion stimulus. These did not occur. (Tables I, III, IV and VI.) When bronchodilatation occurred, it probably facilitated the ventilatory response to the CO_2-O_2 gas mixtures.

Although aminophylline, diamox and both together did not increase the responsiveness of the patients studied, they definitely lowered the threshold of response. This, as indicated previously in the description of methods, is reflected by increase in intensity of response at arbitrarily selected levels of arterial pCO_2. (Table VI.) In one of the two patients with moderate emphysema (R. A.) the intensity of this response was greater with diamox than with aminophylline; and with both drugs together, it was somewhat less than the sum of the individual increases. (Table VI.) In one of the two patients with advanced emphysema (M. A.) there was a significant decrease in threshold only with aminophylline and in the other patient (G. R.) there was no change with either aminophylline or diamox. When the drugs were administered together (Table VI), they produced a highly significant and synergistic response in both of these patients.

The mechanism by which aminophylline stimulates respiration has not been clearly established. It is probably not due to the accumulation of a cerebral metabolite [*21*]. In normal subjects, intravenously administered aminophylline is associated with cerebral vasoconstriction, decrease in cerebral blood flow and internal jugular vein oxygen content [*22,28,29*]. Mean cerebral pCO_2 also likely decreases, since jugular vein pCO_2 and (H^+) ion concentration remain unchanged in the presence of a decrease in arterial pCO_2 [*21*]. According to Stroud et al. [*21*] if aminophylline were to stimulate the respiratory centers directly, it should lower both cerebral venous and arterial pCO_2. Our findings suggest that reduction in the level of plasma $BHCO_3^-$ and possibly of arterial pCO_2 following administration of diamox enhances the ventilatory stimulating action of aminophylline. (Table VI.) This is consistent with the view that aminophylline probably alters the response of the respiratory centers in normal subjects to carbon dioxide or secondary stimuli [*21*].

Our studies were carried out during a falling plasma aminophylline level which might account for the failure to detect an increased sensitivity of response to the arterial pCO_2–(H^+) ion stimulus. This does not appear likely, however, since recent studies in normal subjects demonstrated that with a constant plasma aminophylline level maintained by supplementary infusion following intravenous injection of approximately 0.24 gm. or 0.48 gm. doses (3 and 6 mg./kg. body weight), there was no increased sensitivity, but there was a lowered threshold of response [*21*]. The increase in response of these normal subjects at the arbitrarily

selected 46 mm. Hg arterial pCO_2 level was twice as great with a 6 mg./kg. dose of aminophylline as with a dose of 3 mg./kg. The absolute intensity of response of our patients cannot strictly be compared with those in these normal subjects because of important differences in protocol. In addition to differences in doses of aminophylline injected, our ventilatory and arterial pCO_2 measurements were carried out between the twenty-sixth and thirtieth minutes of inhalation of the CO_2–O_2 mixtures rather than between the eighth and thirteenth minutes, in order to attain a steady state as indicated by the studies of Nielsen [*30*]. The response of each of our patients is comparable in the different study periods, because the doses of aminophylline and of diamox and time sequence were consistent.

The effect of carbonic anhydrase on electrolyte distribution in the central nervous system and its function other than in the formation of cerebrospinal fluid, have not been clarified to date [*31*]. Carbonic anhydrase inhibition by diamox is associated with a prompt decrease in cerebrospinal fluid flow and pressure [*32*]. There is evidence, according to Berliner and Orloff [*31*], which suggests that it is effective in reducing the incidence of convulsive seizures through direct action on the brain. Studies previously reported from our laboratory on the respiratory effects of carbonic anhydrase inhibition by diamox based on Gray's concepts of the integrated regulation of respiration in normal subjects, indicate that it stimulates the respiratory regulatory mechanism either directly or indirectly [*10*]. The present studies confirm the respiratory stimulating action of diamox and indicate that this is associated with a lowered threshold of response to the arterial pCO_2–(H^+) ion stimulus.

SUMMARY AND CONCLUSIONS

Studies have been carried out on the effect of intravenous aminophylline 0.5 gm. (6.9 to 10.9 mg./kg.) and of orally administered diamox (2.9 to 7.2 mg./kg.) every twelve hours, alone, and together with aminophylline, after the metabolic effects of diamox had stabilized, on the respiratory response of four patients, two with moderate emphysema and two with advanced emphysema, to arterial pCO_2–(H^+) ion stimulation induced by inhalation of CO_2–O_2 mixtures.

The sensitivity of response was normal in the patients with moderate emphysema and decreased in the patients with advanced emphysema. None exhibited a significant increase in sensitivity with the drugs even when they caused significant or highly significant reductions in the level of arterial pCO_2, plasma $BHCO_3^-$ and arterial pH. The responsiveness of one patient with moderate emphysema was apparently restricted by mechanical limitations of breathing during the drug periods.

In the other patient with moderate emphysema the intensity of respiratory response at an arbitrarily selected level of arterial pCO_2 increased approximately two, three and four-fold with, respectively, diamox, aminophylline and both administered concurrently. One of the two patients with advanced emphysema exhibited a significant increase following aminophylline, neither did with diamox and in both the response with diamox and aminophylline together was significantly greater than the additive responses of each administered separately. Prior reduction in the level of plasma bicarbonate and arterial pCO_2 induced by diamox may account for the enhanced response to aminophylline.

These observations have certain therapeutic implications. The fact that diamox and aminophylline together increase the ventilatory response of patients with advanced emphysema to the arterial pCO_2– (H^+) ion stimulus suggests that they may be effective in prevention and treatment of CO_2 retention and respiratory depression exhibited by some of these patients during inhalation of oxygen-enriched air. The continued administration of these drugs may also delay or even prevent the development of abnormal regulation of respiration in patients with chronic pulmonary emphysema.

Studies were also carried out on the immediate effects of aminophylline, diamox and both together on minute ventilation, respiratory gas exchange, oxygen uptake, alveolar pO_2, acid-base balance and oxygen saturation of arterial blood hemoglobin, maximum breathing capacity and vital capacity. There was some variation in sequence and intensity of response during five to seven minutes of intravenous injection of aminophylline and over the following forty minutes of observation. When aminophylline was administered together with diamox the changes were more marked. At the end of the forty-five-minute period of observation, moderate ventilatory stimulation was generally present, alveolar

pO_2 and arterial blood hemoglobin oxygen saturation increased, arterial pCO_2 at or several mm. Hg below control level and arterial pH reflected the trend in arterial pCO_2 since plasma $BHCO_3^-$ level did not change. In some instances the reduction in the level of arterial pCO_2 following administration of aminophylline was sufficient to compensate partially the metabolic acidosis induced by diamox.

Acknowledgments: The authors wish to thank Mr. Lewis Burrows, medical student at the New York University College of Medicine, Vincent H. Hollis, Jr., Eva Landesman and Mary A. Homer for their assistance.

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The Role of Systemic Blood Pressure in Cerebral Circulation in Carotid and Basilar Artery Thromboses*

Clinical Observations and Therapeutic Implications of Vasopressor Agents

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INVESTIGATIONS of cerebral circulation show a parallel relationship between systemic arterial pressure and cerebral blood flow [*1*]. Carbon dioxide and oxygen contents of the blood and the carotid sinus reflexes play a role in regulating cerebral circulation when there is an alteration in systemic pressure. Recently we have had an unusual opportunity to study in detail the precise relationship of body blood pressure to brain circulation in two patients in whom cerebral blood flow was impaired by obliterative disease of the carotid or basilar systems. Although neither patient was known to have any great degree of hypertension, a decrease in blood pressure to what would still be considered normotensive levels resulted in the clinical picture of cerebral ischemia. By the use of vasopressor agents, the minimal level of systemic arterial pressure necessary to overcome cerebral ischemia was maintained, and the cerebral symptoms were significantly reduced.

CASE REPORTS

CASE 1. J. L., a sixty-year old carpenter, was admitted to the hospital on April 6, 1955. Two weeks previously he had awakened in the early morning to find upon attempting to get out of bed that his right extremities were numb and paralyzed. The events of this hour were clear in his memory, he was not confused, had no headache, and was able to talk to himself, recognizing no defect in his speech. Within thirty minutes muscle power and sensation began to return and at daybreak, six hours after the onset of symptoms, he felt entirely well. During one year prior to this experience he had had periods of lightheadedness upon arising in the morning which would subside after a period of ambulation. He had noticed blurring of vision six years before this episode. There was no recent change in his vision. Also, during the two years, 1953 to 1955, he had been troubled with periodic bouts of aching pain between the shoulders and in the epigastrium. These pains began one to two hours after meals and persisted for one hour or were relieved by ingestion of milk.

The patient was 5 feet 2 inches tall and weighed 132 pounds. His temperature was 97.6°F., pulse rate 80/min., blood pressure 200/90. His general appearance was in keeping with his age. Memory and mentation were intact. Skin was dry and pale, lips slightly dusky. There was an arcus senilis in both eyes. The vessels in the fundi showed narrowing, tortuosity and moderate A-V nicking. There was a posterior subcapsular cataract on the right. Visual acuity was 20/20 in both eyes. Peripheral vessels were palpably sclerotic although pulses were adequate. A thrill was palpable over the right common carotid artery about 2 inches above the clavicle and at this point a loud bruit was audible. Pulsations of the left carotid were palpably diminished. A bruit was faintly audible over the left malar eminence. There was bilateral high tone hearing loss. The right nasolabial fold was slightly flattened, strength was equal in the extremities, but muscle tone was slightly increased in the right lower extremity. The tendon reflexes were symmetrically active, responses to plantar stimulation were normal, and there were no sensory defects. There were rales in the right chest posteriorily. The heart was not demonstrably enlarged and sounds were distant at the base. A grade 1 systolic murmur was heard at the apex. The liver was slightly enlarged. There were no other significant physical findings. The clinical picture was thought to be consistent with thrombosis of

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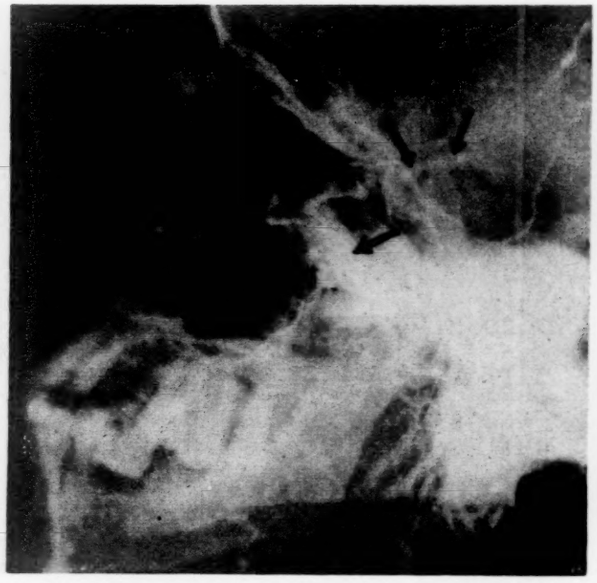

FIG. 1. Injection of 10 cc. of 35 per cent diodrast into left common carotid artery resulted in filling the external carotid, a cluster of vessels behind the orbit (arrow to left), the ophthalmic artery, (middle arrow) and the intracranial portion of the internal carotid artery (right arrow). (Case 1.)

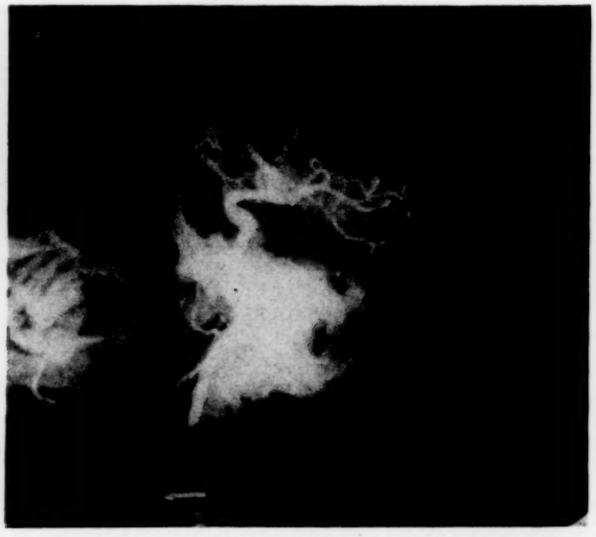

FIG. 2. Injection of 10 cc. of 35 per cent diodrast into right common carotid demonstrated filling of internal and external carotid arteries. Arrow at bottom points to hourglass constriction of internal carotid artery at site of bruit. (Case 1.)

the left internal carotid artery and an abnormality of the right carotid artery.

X-ray examination of the chest and abdomen disclosed calcification of the aortic knob, abdominal aorta and iliac vessels. There were also calcifications of the spleen. The heart was not enlarged. Films of the skull showed calcification of the internal carotid arteries bilaterally. The pineal gland was calcified and in normal position.

On April 12, 1955, a percutaneous left carotid angiography was performed. (Fig. 1.) The proximal portion of the left internal carotid did not fill. There was filling of the external carotid which fed a cluster of vessels in the orbit. The ophthalmic artery filled from these vessels and the intracranial portion of the internal carotid artery was visualized. There were no untoward effects of the procedure. The findings were considered sufficient evidence of left internal carotid artery thrombosis and the bruit over the left maxilla was believed due to the anastomotic vessels in the orbit. In an attempt to explain the thrill and bruit over the right carotid artery, percutaneous right arteriography was performed on April 27. (Fig. 2.) Two to three minutes after a single 10 cc. injection of 35 per cent diodrast® the patient became pale, pulseless and unconscious. The needle was withdrawn and he was put in the Trendelenburg position. When he regained consciousness several minutes later he was confused and had a left hemiparesis. At that time the blood pressure was 140/80. Several blood pressure measurements prior to the procedure ranged between 200/98 to 160/70. Within forty-five minutes mental symptoms and hemiparesis cleared and he was returned to his bed. Three hours later he reported feeling lightheaded and recognized weakness and numbness of the left extremities. The hemiparesis was more profound than it had been immediately following the arteriogram. The bruit over the right carotid could not be heard nor was there a palpable thrill. Blood pressure was 130/80.

An intravenous drip containing 4 cc. of nor-epinephrine in 500 cc. of 5 per cent glucose in water was begun. Within minutes of the start of the infusion the systolic blood pressure reached 250 mm. Hg. The patient reported clearing of lightheadedness, the left hemiparesis cleared and the bruit over the right carotid again became audible. With the nor-epinephrine infusion running, simultaneous observations were made of the clinical status of the patient, systolic blood pressure, intensity of the carotid bruit and electroencephalographic changes. Periods of relative hypotension were induced by stopping the infusion. The results of the examination are illustrated in Figure 3. A systolic blood pressure of 160 mm. Hg seemed necessary to maintain adequate cerebral blood flow. At lower levels, slow waves appeared over the right hemisphere in the electroencephalogram and there was an increase in left hemiparesis, clouding of mentation, and a reduction in the intensity of the bruit over the right carotid artery. At the completion of this examination a continuous infusion of nor-epinephrine was maintained. It became necessary to increase the nor-epinephrine concentration to 8 cc. per 500 cc. of glucose in water. The rate of infusion was regulated to try to maintain a systolic blood pres-

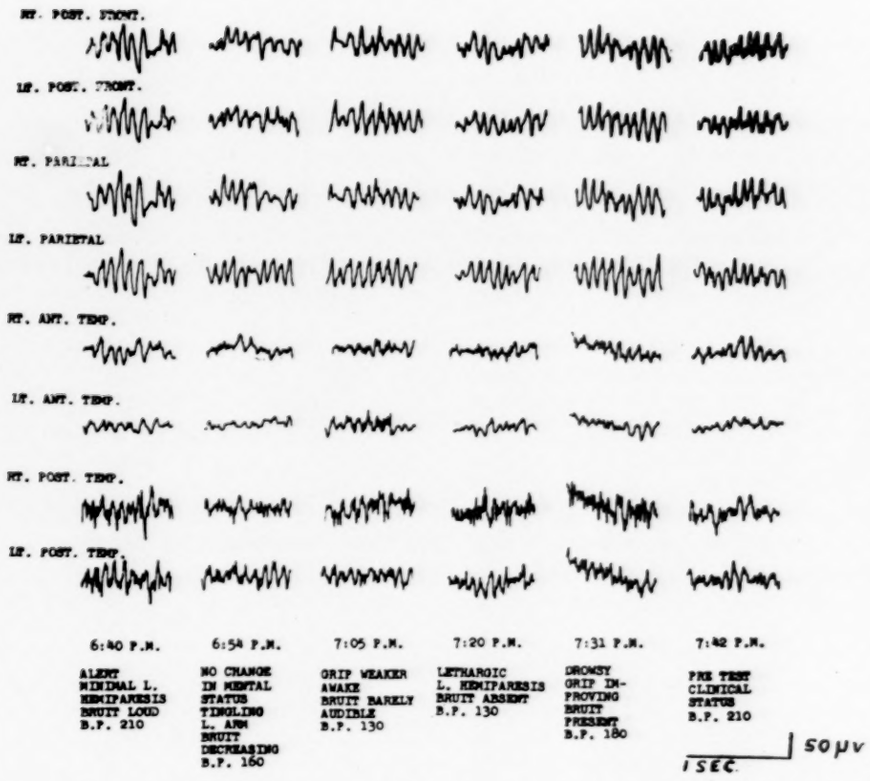

FIG. 3. Case 1. One-second strips of electroencephalogram. Slight decrease in amplitude and slight slowing is seen in right posterior frontal, parietal and posterior temporal areas when systolic blood pressure dropped to 160 mm. Hg. At this time sensory symptoms developed in left hand. At systolic blood pressure of 130 mm. Hg, the left extremities became weaker, patient was lethargic and amplitude remained decreased. When systolic blood pressure was raised to 180 mm. Hg, the amplitude increased and left hemiparesis began to improve.

sure level of at least 160 mm. Hg. On several occasions unavoidable periods of relative hypotension, 140/80, were accompanied by increases in hemiparesis and periods of confusion and hallucination. Elevation of the pressure to systolic levels of 160 mm. Hg was not so dramatically effective as it had been initially in restoring mental function and motor power, although there always was some noticeable improvement.

On April 29, acute pulmonary edema developed. The infusion rate was slowed and the patient was digitalized and given mercurial diuretics. Because of the limitation of fluids that could be given, systolic blood pressure could be maintained only at 135–155 mm. Hg. He became confused and had a moderate left hemiparesis. By May 1, the blood pressure had risen to 200/110. He was awake and active but intermittently irrational. Later that day the infusion infiltrated and the blood pressure dropped to 110/60. He became lethargic and difficult to arouse, but once again was awake and active when a systolic level of 200 mm. Hg was restored. The symptomatic response to a rise in blood pressure lagged about ten minutes behind the actual elevation of the pressure itself. Gradually the infusion rate was decreased. On May 8, with systolic blood pressure levels of 130 mm. Hg, his mental status was improving and he had only mild weakness of grasp in the left hand. The infusion was discontinued and he was placed in the Trendelenburg position. At this time blood pressure ranged between 90–114/50–70. He was alert, cheerful and rational during the day but at night he had hallucinations intermittently and screamed profanely. His left upper extremity was held in a flexed position and resisted passive extension. The left lower extremity was only slightly weak. On May 9 and 10, the head was elevated to a horizontal position. He remained alert in spite of a blood pressure of 110/60, but became confused when his head was elevated. By May 15, he was able to sit in a chair for a few minutes but was again confused in this position if it was more prolonged. His mental status cleared when he was put back flat in bed. Blood pressure at this time was 110–118/60–80 and it did not change significantly when he sat up. By May 20, he was able to sit up for an hour without any ill effects. He slowly regained use of the left upper

extremity. On May 24, a stellate ganglion block was performed on the right side without noticeable improvement. The spinal fluid pressure on May 25 was 86 mm. The cerebrospinal fluid was clear, colorless and acellular, with a total protein of 23 mg. per cent. At this time moniliasis of the mouth developed and he became depressed, truculent, and intermittently confused, but there was no increase in hemiparesis or significant change in blood pressure. By June 8, blood pressure had stabilized at 140/70. Ischiorectal abscess developed and with it a temperature of 105°F. During the period of fever he was disoriented, irritable and confused. In the ensuing week he became increasingly confused. The blood pressure ranged between 115–130/70–85, the bruit over the right carotid remained audible, and the hemiparesis did not increase. His mental status improved when the abscess was surgically drained. On June 14, he was found to have an anemia (hemoglobin 28 per cent). As the anemia was corrected by transfusion and the abscess healed, his mental status improved further and he was able to walk on June 27. His blood pressure was 140/60.

On August 17, the left internal carotid artery was surgically explored. It was found to be a fibrous cord, devoid of lumen. The sheath was stripped. Following this procedure the patient reported less lightheadedness in the morning and felt he had better use of the left hand. His grip became equal to the right hand but there was about 10 to 20 per cent loss of cortical sensibility.

He was dismissed from the hospital November 2, 1955, and was last seen in May, 1956. He recognized difficulty with recent memory and quick wit. This was also obvious to his friends. There was no weakness in the extremities but slight loss of cortical sensation in the left hand persisted. His blood pressure ranged from 160–180/80–90. The bruit over the right carotid was loud. The bruit over the left maxilla could no longer be heard.

Comments. In attempting to reconstruct the hemodynamics of this case we have assumed that the left common carotid artery had been partially or totally occluded prior to the time right hemiparesis occurred in March, 1955. Three weeks later there was arteriographic evidence of extensive collateral circulation through the external carotid and ophthalmic arteries. With the possibility of additional blood flow from the right internal carotid and basilar systems, the total brain circulation remained adequate when systemic blood pressure varied between 160–200/70–98 mm. Hg. The left hemiparesis, which developed after the right carotid angiogram, might have been in part attributed to diodrast toxicity. However, the subsequent correlation of systemic blood pressure levels with change in the bruit, mental status and hemiparesis clearly indicates the role of systolic pressure in total cerebral blood flow. The clouding of consciousness during hypotensive periods suggests that circulation to both hemispheres was insufficient. The right common carotid artery was ineffective. There remained only the collateral from the left external to the internal carotid and the basilar artery. The appearance of the left hemiparesis indicated that there was insufficient collateral circulation to supply adequately the right hemisphere. The reappearance of the bruit and improvement in the hemiparesis at systolic blood pressure levels of 160 mm. Hg indicated that this was necessary to push blood through the constriction in the right common carotid artery at a flow sufficient to cause the bruit. It is interesting that adequate cerebral blood flow was maintained at much lower systemic blood pressure levels, 130 mm. Hg systolic, after the patient had been protected against the harmful effects of systemic hypotension for eleven days. It required fifty days in addition before he was able to walk and had a blood pressure of 140/80. During that period there were symptoms resembling those of postural hypotension; however, there was actually no change in systemic blood pressure when these symptoms occurred. Fever and anemia had effects similar to those seen when his head was elevated during this period.

The electroencephalographic changes in this case were not nearly as striking as the clinical change in the patient. It is believed from these observations that the electroencephalogram does not reflect the severity of circulatory embarrassment which may be present.

The findings in this case suggest further that when there is occlusive disease of the carotid arteries in the neck, a cerebral infarction occurs at a critical point in the relationship of the pressure in the arteries of the head to peripheral resistance. This may be on the same side or the side opposite the occluded carotid artery. Also, gradual occlusion of the main vessels allows for the development of extensive collateral channels as occurs in other regions of the body. This may contribute to the readjustment of intracranial blood flow to lower levels of systemic blood pressure.

Case II. L. R., a fifty-nine year old right-handed salesman, was admitted to the hospital on March 27, 1956, because of a right hemiparesis. Three weeks prior to hospitalization he suddenly experienced numbness, weakness and unsteadiness of the right upper extremity. These symptoms disappeared after several hours but returned again on the morning of hospitalization and were accompanied by "dizziness," and confusion.

There was a history of acute posterior wall myocardial infarction in November, 1955, from which the patient had recovered. He had been back at work when the difficulty with his right upper extremity began. In 1951, he had been hospitalized because of dizziness and confusion. There were no focal signs at that time and symptoms cleared spontaneously.

When examined in the hospital it was noted that there were fluctuations in his level of consciousness.

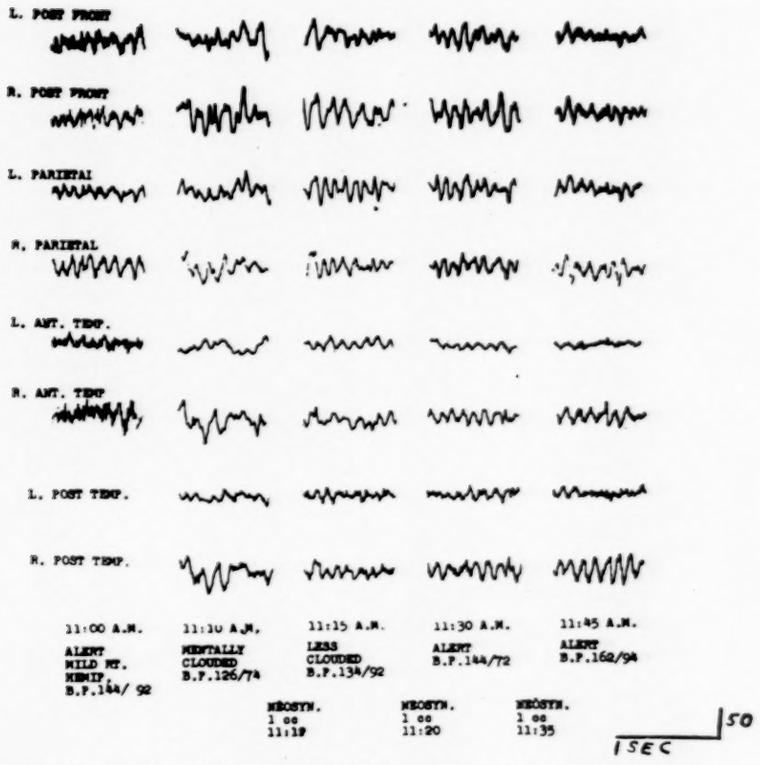

FIG. 4. Case II. One-second strips of electroencephalogram. The correlation of blood pressure changes, physical findings and electroencephalogram alteration is again demonstrated over the right hemisphere.

He was observed for a period of an hour. At times during that interval he would become unresponsive, his eyes were seen to roll, there was no visual fixation, respirations had a sighing quality, and there was frequent yawning. He did not move his extremities upon request nor were there spontaneous movements. However, there was sagging of the right lower face and decreased tone in the right extremities. Blood pressure during these intervals was 120/80. After approximately ten minutes in this state he aroused spontaneously and was able to express thoughts, although speech was slurred. He moved his extremities upon request. There was a right hemiparesis, more severe in the upper extremity and face, and less severe in the lower extremity. During the more lucid intervals his blood pressure was 140/85. This sequence repeated itself several times during the hour of observation. His pulse rate remained stable at 80. In addition to the observation on the state of consciousness and weakness of the right extremities, it was noted that the left carotid pulsation was less forceful than the right. There was arteriolar narrowing in both fundi, most marked in the left. His heart was enlarged to percussion. A_2 was louder than P_2. There was regular sinus rhythm and no murmurs were present. There were rhonchi at the base of the left lung. The remainder of the physical examination revealed no other abnormalities. Soon after admission an electrocardiogram was taken and a spinal puncture performed. The electrocardiogram showed changes consistent with an old posterior myocardial infarct, but no evidence of acute changes. The spinal fluid was clear, colorless and under normal pressure. There were 3 red blood cells per cu. mm. The total protein was 34 mg./100 cc.

Because of our experience with the patient described in Case I, it was decided to administer vasopressor agents to maintain systolic blood pressure above 140 mm. Hg. Intramuscular doses of neo-synephrine® were begun on the afternoon of March 27. Blood pressure was recorded at fifteen-minute intervals and 10 mg. doses of neo-synephrine were administered as necessary, usually at one-half to one hour intervals. Blood pressure was maintained at levels varying between 140–160/80–100. This prevented periods of unresponsiveness. On the morning of March 28, he remained awake, without fluctuations in awareness; the right hemiparesis was still present. An electroencephalographic examination was performed and the blood pressure was allowed to drop to 126/74 by withholding neo-synephrine. He again became mentally clouded and less responsive. Within ten minutes after the administration of neo-synephrine his blood pressure rose to 144/72 and he again became more alert. Concurrent with the hypotensive period there was a change in the electrocephalogram with a de-

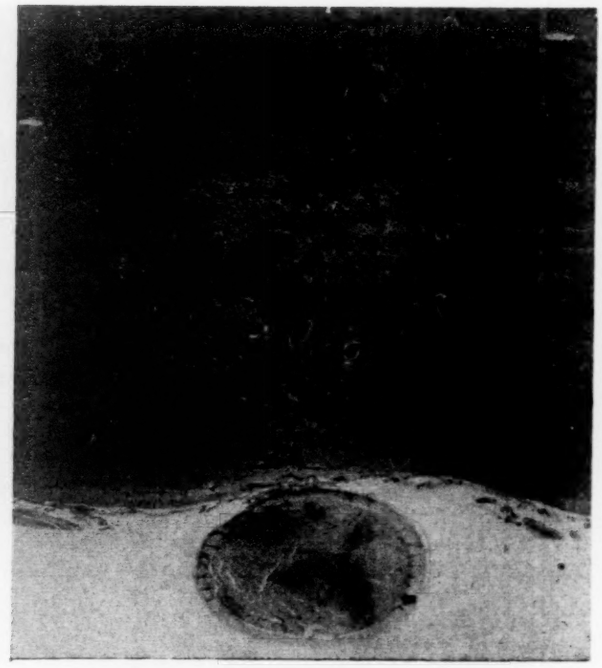

FIG. 5. Photograph of section of pons and basilar artery. The basilar artery is terminally completely occluded by a partially organized thrombus. The vessel wall is thickened. Original magnification, × 15. (Case II.)

pression of amplitude on the left side and the appearance of high voltage slow waves over the right side. (Fig. 4.) Subsequent to this examination the patient was maintained on neo-synephrine as needed to sustain systolic blood pressure above 140 mm. Hg. This was successful for the remainder of the day and he expressed the feeling that his head was clearing. He had increased strength in the right extremities and was noticeably more alert. In the evening of March 29 it became more difficult to maintain blood pressure with neo-synephrine. He required 10 mg. at ten- to fifteen-minute intervals and continuous singultus developed. He became flushed and sweaty and there were periods of hypotension (90/50) as well as excessive rises to 210/140. That evening infusion of norepinephrine was initiated. It was no longer possible to maintain a constant blood pressure level. There were periods of shock with blood pressure 40/0, and these were followed by excessive elevations to 200/140. His condition began to deteriorate. He became increasingly unresponsive. By midnight of March 30 he reached a period of irreversible hypotension. Systolic levels were below 80 and the diastolic was unobtainable. He died at 4 A.M. on March 31, 1956.

Postmortem examination on the cerebral vascular system disclosed an occlusion of the basilar artery by a soft, gray-yellow clot. (Fig. 5.) The older part of the clot was close to the site of the exit of the superior cerebellar and posterior cerebral arteries; the more recent portion extended toward the vertebral arteries. The

FIG. 6. Photomicrograph of section of pons demonstrating neuronal degenerative changes. Hematoxylin and eosin stain; original magnification, × 200. (Case II.)

right vertebral was relatively normal. The left was markedly arteriosclerotic and the lumen reduced to pinhole size. At the left occipital pole there was a recent hemorrhagic softening, confined mainly to the cortex. There were dilated small blood vessels in the pons. The remainder of the gross examination of the brain revealed no abnormalities. The microscopic findings of the brain stem sections indicated that the lesions were of very recent origin, there being degenerative changes in the neurons without cellular inflammatory reaction. (Fig. 6.) Gross and microscopic examination of the heart indicated no recent myocardial damage.

Comment. It is our assumption that collateral flow from the carotid into the vertebral systems was blocked by the thrombus at the bifurcation of the basilar artery. Thus the basilar blood flow was entirely dependent upon the vertebral arteries. The improvement in symptoms during the period of induced hypertension was affected by increased flow through diseased vessels of the vertebral system. The terminal events, therefore, were a reflection of progressive occlusion of the basilar artery itself. This is in keeping with the histologic appearance of the pons and the fresh thrombus in the basilar artery. The failure of vasopressor agents to maintain blood pressure may have been due to brain stem disease.

COMMENTS

The possible harmful effects of vasopressor agents on cerebral blood flow has been inferred from studies in normal patients [*2,3*]. Although vasopressor drugs are capable of producing constriction of cerebral blood vessels under normal conditions, the amount of vascular narrowing that can occur in arteriosclerotic arteries is prob-

ably limited because of the rigidity of the vessel itself and because of the destruction of its contractile tissue by the atherosclerotic material.

Another mechanism by which nor-epinephrine and similar compounds might adversely affect cerebral blood flow would be by stimulation of the carotid sinus. It has an indirect effect by regulating systemic arterial pressure, but in addition it may have an effect on cerebral circulation directly [*4,5,6*]. The work of Heymans, Delaunois and Martini [*7,8*] has shed more light on the exact mechanisms by which the carotid sinus produces its effect. Their experiments indicate that it is the state of resistance and distensibility of the arterial wall of the carotid sinus itself which is responsible for the pressor effects. The receptors within the carotid sinus are stimulated by stretch, and therefore increased arterial pressure exerts its effect not by direct stimulation of the pressoreceptors but reflexly by stretching the vascular wall in which they are contained. Thus a carotid sinus which is made more rigid by atherosclerosis or thrombosis can be expected to react less to an increase in intra-arterial pressure. On the other hand, the nerve endings may continue to respond to direct external mechanical stimuli such as carotid sinus massage and might result in an oversensitive sinus, causing episodes of hypotensive syncope. Actually, such an idea was advanced as early as 1927 by Hering [*9*] who suggested that atheromatous changes in the walls of the aorta and sinuses might interfere with the transmission of intravascular pressure to the sensory nerve. All this evidence suggests that a diseased carotid sinus may fail to function in a normal fashion and any anticipated adverse effects on cerebral blood flow, exerted directly or indirectly on the carotid sinus by vasopressor drugs, may not occur.

The possibility that nor-epinephrine might produce complete obstruction to the flow of blood through an already narrowed carotid vessel by constricting the artery even further must be considered, but here again we do not know that such vasoconstriction can occur in an atherosclerotic carotid artery. Some experimental work in the pathogenesis of death due to x-ray exposure [*10*] is of interest here. It was observed that carotid arteries of radiated dogs failed to exhibit constriction following stimulation by epinephrine and nor-epinephrine. Histologic examination of these arterial segments showed degenerative changes of the muscle elements. Although the study is not directly applicable to our cases, it is further evidence that diseased arteries may not be able to constrict when stimulated by vasoconstrictor drugs.

It is common practice to attempt to correct hypotensive states by the use of potent vasopressor agents, but such drugs are not generally employed unless the systolic blood pressure falls below a level of 100 mm. Hg. Since the widespread use of potent antihypotensive drugs, such as the ganglionic blocking agents, there has been a growing interest in the cerebrovascular changes which may occur during a state of "relative" hypotension. Very few studies have been done, however, to accurately delineate the critical level of relative hypotension below which definite brain damage occurs. Recently a technic has been described for testing insufficiency of the basilar and carotid arteries by lowering blood pressure with a tilt table and recording electroencephalographic changes [*11*]. It appeared that an induced fall of blood pressure averaging 43/19 mm. Hg may give rise to focal abnormalities, with slowing of the electroencephalogram, dizziness, confusion and episodic syncope. It would appear then that in a patient with obliterative disease of the carotid or basilar arteries the amount of blood which will flow beyond the narrowed blood vessel into the brain will depend directly on the head of pressure of the blood column. Actually this proved to be the case in our two patients. The patient with carotid thromboses proved to have a critical systolic arterial pressure of 160 mm. By maintaining a pressure above this with nor-epinephrine there was clinical evidence of improved cerebral function, a reflection of cerebral blood flow. In the second case of basilar thrombosis, neo-synephrine was temporarily effective in improving the clinical picture by elevating the blood pressure, but this was followed by an episode of hypotension and the patient died.

A final word should be said about the possible beneficial effects of "stripping" of the carotid sheath. Our patient appeared to benefit from this denervation procedure and we have subsequently performed the operation in two other cases, only one patient benefited from it. The effectiveness of this type of sympathectomy deserves further evaluation in other cases.

SUMMARY

The role of systemic blood pressure in maintaining cerebral blood flow has been demon-

strated in the cases of two patients with advanced arteriosclerotic disease of the carotid and basilar arteries.

In one patient a minimum systolic pressure of 160 mm. Hg was necessary to prevent or improve focal neurologic symptoms. In the other, the critical level was 140 mm. Hg. These levels, maintained by potent vasopressor drugs, were considered the minimum necessary to sustain adequate cerebral blood flow. The hemodynamics, mechanisms of infarction, and their therapeutic implications are discussed.

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Sequence of Ventricular Contraction in Human Bundle Branch Block*

A Study Based on Simultaneous Catheterization of Both Ventricles

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EXPERIMENTS in which the two main bundle branches were sectioned in the dog [*1–3*], the monkey [*4*] and in the revived, perfused human heart [*5*] have led to the present concept of bundle branch block. In accordance with this concept, there is complete interruption of impulse conduction in one bundle branch of the patients who satisfy electrocardiographic criteria for complete bundle branch blocks. Transseptal depolarization takes place from the normal bundle branch and the impulse excites the affected bundle below the site of the block. In experimental bundle branch block, the free wall of the ventricle on the interrupted side is depolarized approximately 0.04 second after the contralateral ventricle. This represents the time required for trans-septal depolarization [*1,3*].

It has been demonstrated that myocardial excitation precedes myocardial contraction [*6*]. Therefore, if depolarization of one ventricle is delayed, as has been presumed to occur in patients with the electrocardiographic configuration of complete bundle branch block, a delay in the onset of contraction of that ventricle would be expected. Such delay in the onset of ventricular contraction has been clearly demonstrated in ventricular pressure curves obtained from dogs with experimentally produced complete bundle branch block [*7*]. The recent development of methods for the recording of pressure pulses from both ventricular cavities in man has now made it possible to examine critically the validity of the classic bundle branch block concept.

METHODS

Right heart catheterization was performed in the usual manner. With the right heart catheter in the pulmonary artery, transbronchial left heart catheterization was then carried out [*8*]. After the transbronchial introduction of a needle into the left atrium, a polyethylene catheter was advanced through this needle into the left ventricle. The right heart catheter was then withdrawn into the right ventricle and both ventricular pressure pulses were recorded together with lead II of the electrocardiogram.

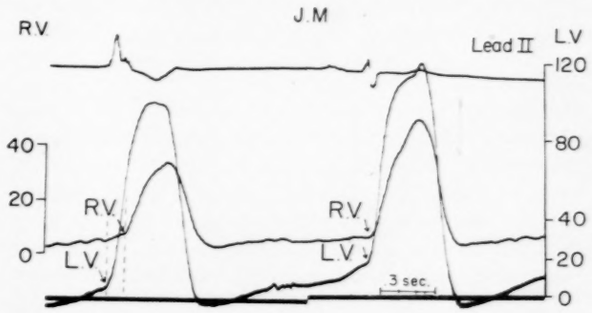

FIG. 1. Simultaneous right ventricular (R. V.) and left ventricular (L. V.) pressure pulses in a patient with normal intraventricular conduction. The beat on the right is normally conducted and the onset of left precedes the onset of right ventricular contraction by a normal interval (0.010 second). The beat on the left is a left ventricular premature contraction in which the onset of left ventricular contraction precedes the onset of right ventricular contraction by 0.100 second. Arrows indicate onset of contraction.

Cournand catheters and a Statham P23A strain gauge were employed for the right heart catheterization while a Statham P23D gauge was used for transducing left ventricular pressures. The delay in transmission of a pressure pulse through both catheter-strain gauge systems was measured [*9*] and found to be equal (0.006 second). A multichannel, cathode ray, photographic recorder,† in which possible errors of

† Manufactured by Electronics for Medicine, Inc., New York, N. Y.

* From the Laboratory of Cardiovascular Physiology and the Clinic of Surgery, National Heart Institute, Bethesda 14, Md. Presented in part at the 29th Annual Scientific Session of the American Heart Association, Cincinnati, Ohio, October 27, 1956.

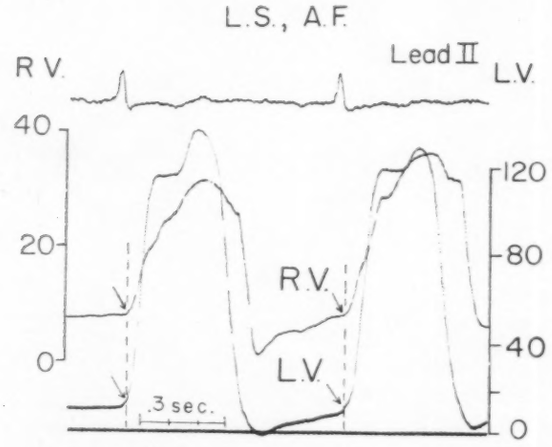

FIG. 2. Simultaneous right ventricular (R. V.) and left ventricular (L. V.) pressure pulses in a patient with atrial fibrillation and normal intraventricular conduction. The L. V. begins to contract 0.015 second before the R. V.

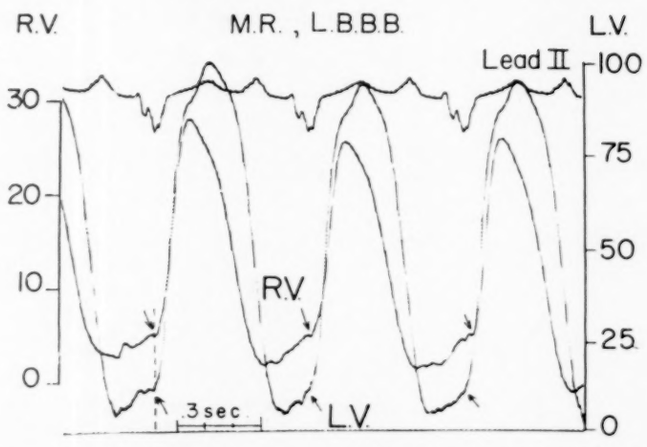

FIG. 3. Simultaneous right ventricular (R. V.) and left ventricular (L. V.) pressure pulses in a patient with the electrocardiographic configuration of complete left bundle branch block with a QRS duration of 0.14 second. The onset of left ventricular contraction precedes the onset of right ventricular contraction by 0.010 second.

parallax are eliminated, was employed. The paper speed was 75 mm./second.

In four patients with interatrial septal defects, catheterization of the right side of the heart was carried out by way of the saphenous vein; the catheter was then advanced through the defect into the left atrium and left ventricle. Right and left ventricular pressures were recorded in rapid succession together with a reference electrocardiographic lead. In these patients the temporal relationship between the onset of contraction in the two ventricles could be calculated by measuring the time interval from the onset of the QRS complex to the onset of the rise of pressure in each ventricle.

Measurements were carried out to the nearest .005 second and were averaged for ten beats. The delay in the transmission of the pressure pulse was taken into account by subtracting 0.005 second from all measurements between the onset of electrical and mechanical events.

Validation of the Method. It is conceivable that in the presence of ventricular asynchronism the earlier contracting ventricle displaces the interventricular septum toward the opposite ventricle. In this manner, the pressure in the delayed ventricle could rise before the actual onset of its contraction. This would falsely indicate the simultaneous onset of ventricular contraction. It was therefore considered that a demonstration of asynchronous ventricular contraction would be pertinent.

The ventricle from which an extrasystole arises is depolarized before, and therefore contracts before the contralateral ventricle. Extrasystoles were produced in patients with normal intraventricular conduction by tapping the epicardium of patients undergoing thoracotomy. Simultaneous pressures were measured in both ventricles by means of needle puncture. An extrasystole originating from the left ventricle is demonstrated in Figure 1. The onset of pressure rise in the left ventricle clearly precedes that in the right ventricle.

On the basis of the observations cited, and the demonstration of asynchronous ventricular contraction in experimental bundle branch block [7] and in some patients with electrocardiograms showing right bundle branch block, it is believed that the method of simultaneously recording ventricular pressures is suitable for the detection of asynchronous ventricular contraction.

RESULTS

Normal Intraventricular Conduction. In a previous study [*10*] it was noted that both ventricles began to contract almost simultaneously in human subjects with normal intraventricular conduction; the onset of right ventricular contraction followed that of left ventricular contraction by an average of only .013 ± .0043 second. Since the technics used in the present study differed from those employed in the earlier investigation, the time of onset of ventricular contraction was determined in seven additional patients with normal intraventricular conduction. The results were similar to those previously described; the onset of right ventricular contraction followed that of left ventricular contraction by an average time interval of .012 second. The maximum delay observed was 0.020 second. (Fig. 2.)

Left Bundle Branch Block. Five patients with electrocardiograms showing complete left bundle branch block, according to the criteria of Wilson

TABLE 1

RELATIONSHIPS BETWEEN ELECTRICAL AND MECHANICAL EVENTS IN 15 PATIENTS WITH ELECTROCARDIOGRAMS SHOWING COMPLETE BUNDLE BRANCH BLOCK

Patient	Age (yr.)	Diagnosis	QRS Duration (sec.)	Q → LV_s* (sec.)	Q → RV_s† (sec.)	LV_s → RV_s‡ (sec.)
Patients with Electrocardiograms Showing Complete Left Bundle Branch Block						
M. R.	41	Idiopathic myocarditis	0.14	0.065	0.075	0.010
H. S.	46	Coronary artery disease	0.16	0.065	0.065	0.000
J. R.	17	Congenital subvalvular aortic stenosis, postoperatively	.0.15	0.055	0.055	0.000
L. A.	62	Rheumatic aortic and mitral regurgitation	0.14	0.080	0.080	0.000
M. S.	49	Idiopathic myocarditis	0.16	0.075	0.065	−0.010
Patients with Electrocardiograms Showing Complete Right Bundle Branch Block						
R. B.	41	No evidence of cardiac disease	0.13	0.060	0.100	0.040
J. C.	19	No evidence of cardiac disease	0.12	0.045	0.085	0.040
J. S.	51	Idiopathic myocarditis	0.14	0.055	0.095	0.040
G. W.	49	(?) Ebstein's anomaly	0.12	0.060	0.100	0.040
E. T.	49	Interatrial septal defect	0.12	0.055	0.065	0.010
J. T.	18	Interatrial septal defect	0.12	0.075	0.085	0.010
R. S.	47	Interatrial septal defect	0.12	0.080	0.080	0.000
J. M.	26	Mitral stenosis	0.12	0.055	0.075	0.020
D. M.	46	Interatrial septal defect	0.12	0.055	0.055	0.000
V. T.	37	Interatrial septal defect	0.13	0.075	0.095	0.020

* Q → LV_s = time interval from onset of QRS to onset of left ventricular contraction.
† Q → RV_s = time interval from onset of QRS to onset of right ventricular contraction.
‡ LV_s → RV_s = time interval from onset of left to onset of right ventricular contraction.

et al. [*11*], were studied. In all patients studied, (Table 1) the onset of contraction in the left ventricle occurred within 0.010 second of the onset in the right ventricle. If complete interruption of conduction in the left main bundle existed, the onset of left ventricular contraction would follow the onset of right ventricular contraction by 0.04 second, the time required for trans-septal depolarization.

In four patients the onset of left ventricular contraction either preceded, or was simultaneous with the onset of right ventricular contraction. (Fig. 3.) In one patient (M. S.), the onset of left ventricular contraction followed the onset of right ventricular contraction by 0.01 second. In another patient (L. A.), the time interval between the onset of QRS and of left ventricular contraction slightly exceeded the normal limits [*10*]. It is noteworthy, however, that the onset of contraction in both ventricles was simultaneous in this patient.

Particular attention is called to the findings in a seventeen year old boy (J. R.) with congenital subvalvular aortic stenosis, who was studied both before and after the appearance of the left bundle branch block pattern. (Fig. 4.) Preoperatively, the electrocardiogram showed normal intraventricular conduction, a QRS duration of 0.07 second, with left ventricular hypertrophy and strain; the time interval between the onset of ventricular depolarization and of left ventricular contraction was within normal limits, .050 second [*10*]. During open operation for relief of the subvalvular stenosis [*12*] the left bundle branch block pattern with a QRS duration of 0.15 second appeared, and this abnormality revealed on electrocardiogram has persisted. Three weeks postoperatively, study revealed that the time interval between the onset of ventricular depolarization and of left ventricular contraction remained unchanged, and that the onset of ventricular contraction was simultaneous in both ventricles.

Right Bundle Branch Block. Ten patients whose electrocardiograms satisfied the criteria established by Wilson and associates for the diagnosis

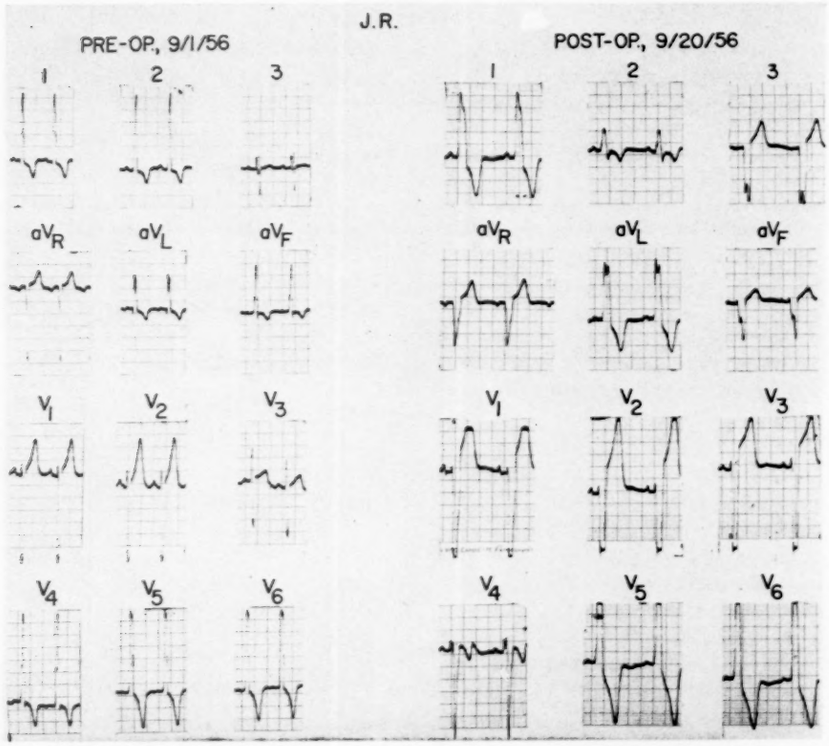

FIG. 4A. Electrocardiograms of a seventeen year old boy with congenital subvalvular aortic stenosis. Preoperatively there is normal intraventricular conduction, left ventricular hypertrophy and strain, QRS = 0.07 second. Postoperatively there is left bundle branch block, QRS = 0.15 second.

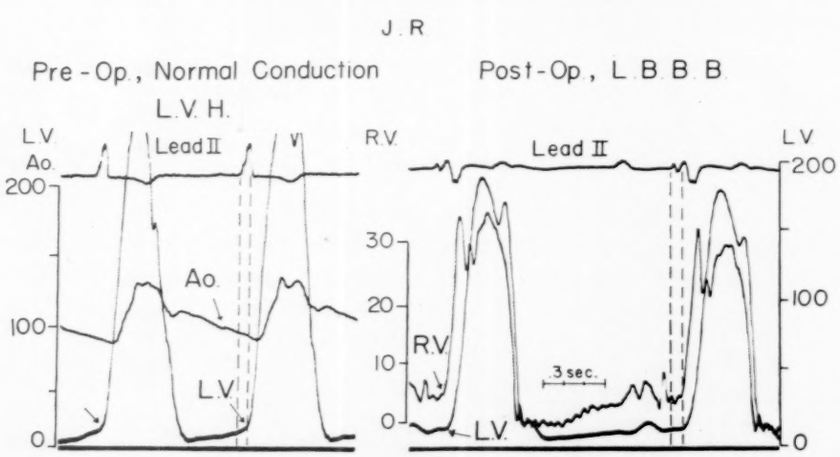

FIG. 4B. Pressure pulses in J. R. whose electrocardiograms are illustrated in Figure 4A. Preoperatively the left ventricular (L. V.) and central aortic (Ao.) pressures were recorded. Postoperatively both ventricular pressures were obtained. The broken vertical lines represent the time interval from the onset of QRS to the onset of L. V. contraction.

of right bundle branch block [*11*] were studied. (Table 1.) Distinct asynchronism, with right ventricular contraction commencing 0.04 second after left ventricular contraction (Fig. 5) was noted in four patients. However, in the other six patients the onset of ventricular contraction was either simultaneous (Fig. 6) or left ventricular contraction preceded right ventricular contraction by a normal interval (up to 0.02 second). In one patient (V. T.), although the time interval

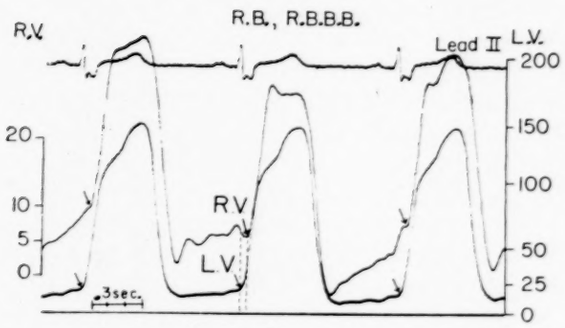

FIG. 5. Simultaneous right ventricular (R. V.) and left ventricular (L. V.) pressure pulses in a patient with the electrocardiographic configuration of complete right bundle branch block with a QRS duration of 0.13 second. The onset of right ventricular contraction follows the onset of left ventricular contraction by 0.04 second.

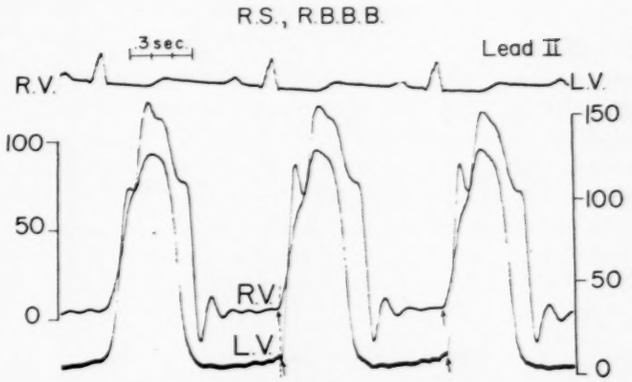

FIG. 6. The onset of contraction is simultaneous in the right ventricle (R. V.) and left ventricle (L. V.) of this patient with the electrocardiographic configuration of right bundle branch block, with a QRS duration of 0.12 second. This patient had an interatrial septal defect and marked right ventricular hypertrophy.

between the onset of QRS and of right ventricular contraction exceeded the normal [*10*], the time interval separating the onset of right and left ventricular contractions was within normal limits.

COMMENTS

Anatomic examinations of the conduction system in man [*13–15*] have demonstrated a striking difference between the two main bundle branches. The right bundle branch is a long thin, ribbon-like structure. In contrast, the left main branch is a short broad band which divides near its origin into numerous fasciculi, some of which arise even proximal to the bifurcation of the bundle of His [*13*]. Thus, a lesion which could produce complete anatomic interruption of the left bundle branch would have to be very extensive. Because of its structure, the right bundle branch seems far more vulnerable to complete interruption of conduction than the left bundle branch.

The classic concepts of bundle branch block are based on observations in experimental animals with complete section of the bundle branches. It has been assumed that patients whose electrocardiograms resemble those noted in the animals with experimental bundle branch block also have complete interruption of conduction in their main bundle branches. There has been, however, some suspicion of the validity of this reasoning [*16–23*]. Segers has supported the view that the electrocardiographic patterns of bundle branch block in man may sometimes result from focal intraventricular conduction disturbances [*16*]. Castro and associates have demonstrated that electrocardiograms in dogs with focal block may resemble electrocardiograms taken of dogs with experimental bundle branch block as well as tracings that have been termed bundle branch block in man [*17*]. In patients with anatomic right ventricular hypertrophy and in whom electrocardiograms presented the right bundle branch block pattern, spatial vectorcardiograms showed the presence of right ventricular hypertrophy but usually gave no evidence of an associated conduction disturbance [*18,19*]. It has also been observed that the time interval between the onset of ventricular depolarization and right ventricular contraction was normal in ten of fifteen patients with clinical evidence of right ventricular hypertrophy in whom electrocardiograms showed right bundle branch block [*20*]. The latter study indicated that in these ten patients complete interruption of conduction in the right bundle branch did not exist.

The use of the cardiac catheter technic in the study of intraventricular conduction disturbances [*24*] has made the precise measurement of the time interval between the onset of ventricular depolarization and of right ventricular contraction possible [*20*]. The present investigation, based on simultaneous catheterization of both ventricles, permitted determination of the temporal relationship between the onset of contraction in both ventricles.

In the four patients whose electrocardiograms showed right bundle branch block and in whom the onset of right ventricular contraction followed that of left ventricular contraction by 0.04

second, the findings are compatible with the hypothesis that complete interruption of conduction in the right bundle branch existed. However, in the other six patients whose electrocardiograms presented the pattern of right bundle branch block, and in all five patients whose electrocardiograms showed left bundle branch block, the onset of contraction in both ventricles was essentially simultaneous. These data indicate that complete interruption of conduction in one of the main bundle branches did not exist in these patients.

In this investigation attention has been focused primarily on the time of onset of ventricular contraction and not on the timing of subsequent events in the cardiac cycle, such as the onset and termination of ejection. However, it appears from the ventricular pressure curves that in the patients whose electrocardiograms showed left bundle branch block, a paradoxic relationship between semilunar valve closure existed [*10*] with aortic valve closure following pulmonic valve closure. Similar information has also been obtained from phonocardiographic investigations in such patients [*25*]. Segers, utilizing electrokymography, has made interesting observations on a patient with the electrocardiographic pattern of intermittent left bundle branch block [*26*]. In the presence or absence of the bundle branch block configuration, the onset of left ventricular contraction was normal. However, the onset of aortic ejection was delayed in the presence of the bundle branch block pattern.

The observations cited may be explained by the following hypothesis: In the patients studied whose electrocardiograms showed complete left bundle branch block, a conduction block may exist in some of the branches of the left main bundle, or within the left ventricular myocardium. Such a conduction disturbance would account for the prolongation and abnormal configuration of the QRS complex and also for the delay in the onset and termination of ventricular ejection. However, since the onset of left ventricular contraction in these patients is normal, a substantial portion of their left ventricle must begin to contract at a normal time and must therefore be depolarized at a normal time. Thus complete interruption of conduction could not be present.

This hypothesis is supported by the investigations of Braun-Menendez and Solari [*7*], who observed that delayed onset of ventricular contraction occurred only in those dogs in which subsequent anatomic examination revealed complete section of the entire main bundle branch. However, in dogs in which no delay in the onset of ventricular contraction was apparent, in spite of the bundle branch block pattern on the electrocardiogram, the bundle branch had not been completely interrupted. Thus incomplete interruption of conduction can lead to the electrocardiographic configuration of complete bundle branch block.

While the considerations presented herein could also be applied to the patients whose electrocardiograms showed right bundle branch block without abnormally asynchronous onset of right ventricular contraction, an alternate explanation is possible. All these patients had lesions which produced anatomic enlargement of the right ventricle. It has been demonstrated previously that the same fundamental vectorcardiogram was present in all patients with right ventricular hypertrophy, regardless of whether the electrocardiogram showed right ventricular hypertrophy or incomplete right bundle branch block [*27*]. In patients with clinical evidence of right ventricular hypertrophy and the electrocardiographic picture of right bundle branch block and normal onset of ventricular contraction, it is possible that the electrocardiogram merely reflects the presence of the right ventricular hypertrophy.

SUMMARY

1. Catheterization of both ventricles was performed in seven patients with normal intraventricular conduction, in five patients with electrocardiograms showing left bundle branch block and in ten patients with the electrocardiographic pattern of right bundle branch block.

2. None of the patients with the electrocardiographic configuration of complete left bundle branch block had a significant delay in the onset of left ventricular contraction. Four of ten patients with electrocardiograms showing complete right bundle branch block had a delay of 0.04 second in the onset of right ventricular contraction. However, in the other six patients no abnormal delay in the onset of right ventricular contraction was noted.

3. The absence of abnormally asynchronous ventricular contraction in patients showing the electrocardiographic picture of complete bundle branch block is not compatible with the concept of bundle branch block which postulates com-

plete interruption of conduction in one of the main bundle branches.

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The Ventricular Gradient in Space*

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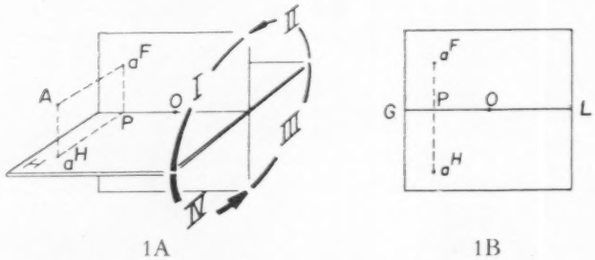
IT has been shown repeatedly [*1–6*] that changes in temperature, blood supply or autonomic balance of the heart are accompanied by alterations in the T wave. Warming the apex of a frog's heart causes a negative T wave to become positive and a positive T wave to increase in size. Cooling the apex has an opposite effect, as does warming the base. It is evident that local differences in temperature can alter the process of regression without modifying that of activation. Drugs, toxic agents, ischemia, anoxemia, pH, electrolytes and endocrine factors exert similar influences.



1A 1B

FIG. 1. A, space is divided into four quadrants by the frontal and horizontal planes. The projection of a point on a plane lies at the foot of a perpendicular from the point to the plane. The point is represented orthographically by its projections. To show these on a single surface, the horizontal plane is rotated about the ground line into the frontal plane, its anterior half moving down; its posterior half, up. B, orthographic representation of point A. aH has been brought below the ground line. OP, Pa^F and Pa^H are the respective Cartesian or XYZ coordinates, of A with reference to a null-point, 0.

The form of the T wave is affected by differences in duration of the excited state in various regions of the myocardium. This was recognized in 1892 by Bayliss and Starling [*7*] who concluded that a positive second "course" indicates a longer persistence of the excited state at the base than at the apex. The vector representing the electromotive force produced by this lack of uniformity was termed the "ventricular gradient" by Wilson [*8,9*], who derived its projection on the frontal plane by area measurement of the bipolar limb leads.

If the duration of excitement were the same in all fibres of the myocardium, the T wave would be not similar but identical with the QRS wave in size and configuration, with reversed polarity, i.e., a mirror-image. The fact that it is not and, in normal subjects, is concordant with QRS, indicates a second force acting during inscription of T, a force of no trivial magnitude.

In the words of Ashman [*10*], "The ventricular gradient is the fundamental quantity in electrocardiography. A change in the gradient means a change in the state of the muscle." Complete identification of its magnitude and spatial orientation is therefore desirable. Considerable work has been directed to this end but as no precise method of spatial analysis has been available only measurements of the frontal plane projection, or manifest gradient, have been compiled. These have limited significance, and evaluation of the clinical usefulness of the gradient based upon such studies is premature. They have, nevertheless, served to sustain interest in this intriguing concept. Recently there have been attempts to measure the gradient spatially. It is proposed here to present a simple geometric method of determining this force as accurately as present day instrumentation allows.

METHOD

In orthographic projection, the frontal (F) and the horizontal (H) planes are considered transparent and intersect at a ground line (GL). F and H form by their intersection four dihedral angles, or quadrants, as in Figure 1A.

A point in space may be represented by its projections on the F and H planes. Each projection lies at the foot of a perpendicular from the point to its respective plane. In Figure 1A, a^F and a^H are the respective F and H projections of point A. To represent A on a single surface, the H plane is rotated about GL as an axis until it is superimposed on the F plane, the portion of the H plane in front of GL falling down and

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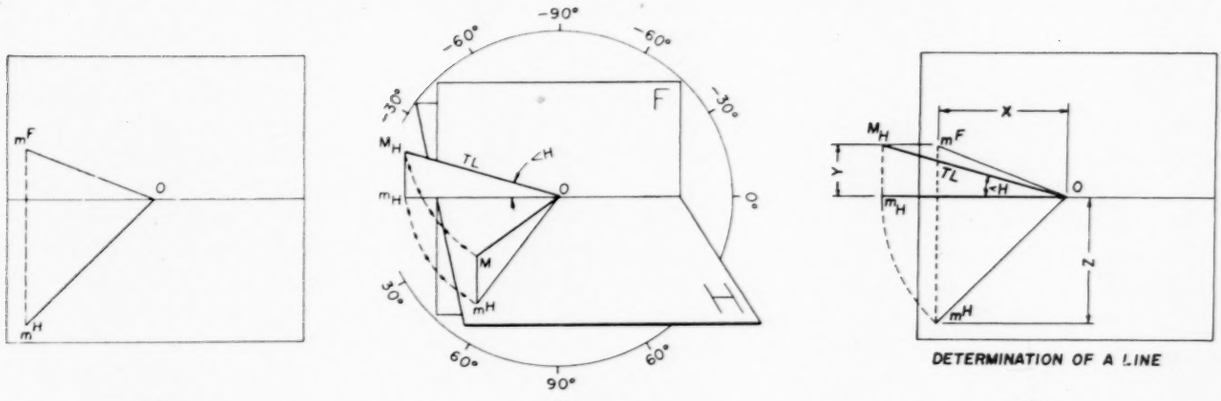


2A 2B 2C

FIG. 2. A, orthographic representation of vector $\overline{OM}$. B, determination of vector $\overline{OM}$. A plane is passed between the vector and its horizontal projection. This is perpendicular to the horizontal plane and is called a horizontal projecting plane. When it is revolved into the frontal plane, the true magnitude (TL) of OM and the angle it makes with the horizontal plane ($<$H) are seen, these having been unaffected by the revolution. C, determination of magnitude and $<$H by orthographic construction. An arc of radius Om^H is drawn intersecting the groundline at m_H. A perpendicular m_HM_H, is erected at m_H to the altitude of m^F. OM_H is then the magnitude (TL) of vector $\overline{OM}$ and $<m_HOM_H$ is the angle it makes with the horizontal plane ($<$H).

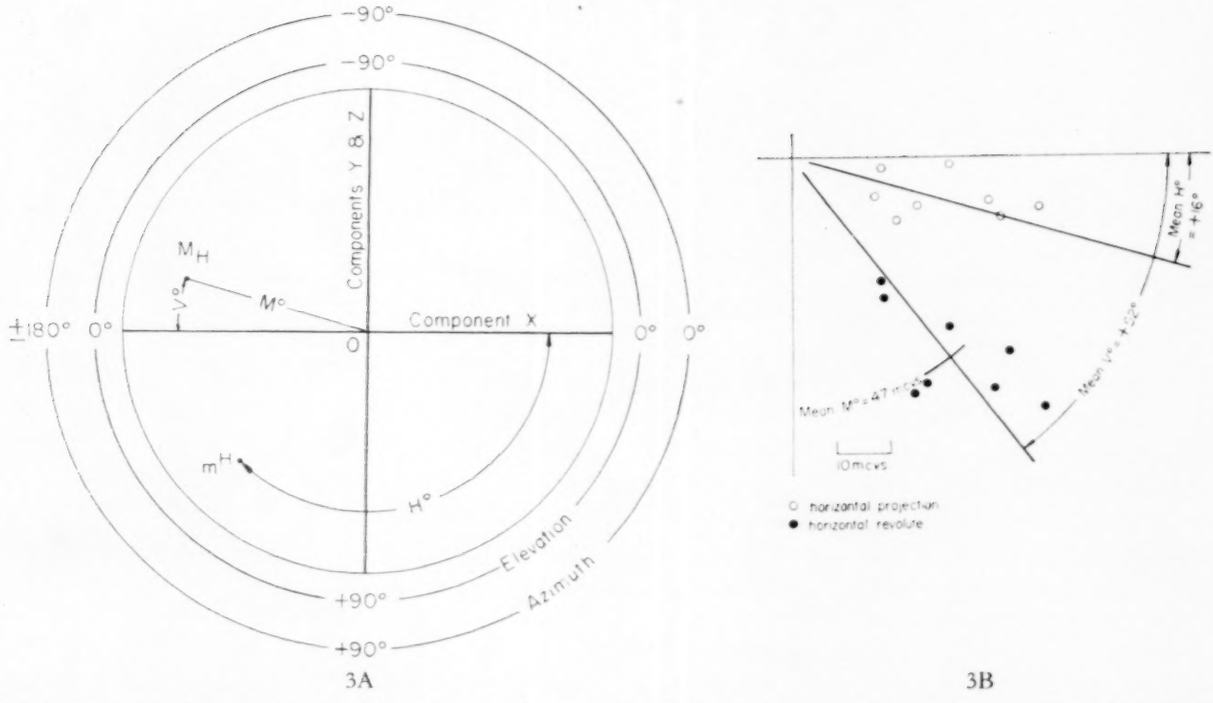
that part in back of GL moving up. In this new position all the plane of paper above GL represents that part of the H plane behind GL as well as the F plane above GL; the part below GL represents that portion of the H plane anterior to GL as well as the F plane below GL. Figure 1B is the orthographic representation of Figure 1A; a^F has not been affected by rotation of the H plane, but a^H has been brought below the ground line. Between them is the interrupted line of recall. It is perpendicular to GL.

It can be deduced from Figure 1 that in orthographic representation A point in I has its F projection above and its H projection below GL; A point in II has both projections above GL; A point in III has its F projection below and its H projection above GL; A point in IV has both projections below GL.

Let us assume the vector $\overline{OM}$ with horizontal projection Om^H and frontal projection Om^F, as in Figure 2A. The problem is to determine its true length (magnitude) and its orientation in space. If we pass a plane between the vector and its horizontal projection, this plane will be perpendicular to H and is called a horizontal projecting plane. This has been performed in Figure 2B. The plane, OMm^H, can then be revolved about O as a pivot, remaining perpendicular to H, until it is superimposed on the frontal plane. The new positions of m^H and M are now m_H and M_H. OM_H is TL, the true length or magnitude, of the vector, and $<M_HOm_H$ is the angle subtended with H by $\overline{OM}$, these having been unaffected by the revolution. Measurements here will not give us the true dimensions but if the revolution is performed orthographically, as in Figure 2C, these are obtained. An arc of radius Om^H is drawn to the groundline, and a perpendicular m_HM_H erected to the altitude of m^F. OM_H is then the true length of $\overline{OM}$, and $<M_HOm_H$ is the angle $\overline{OM}$ subtends with H. This may be designated $<$H. It is the angular distance of $\overline{OM}$ from the horizontal plane, or its elevation. Magnitude and elevation are the polar coordinates of the vector terminus, M, on the horizontal projecting plane. The position of the plane is its azimuth. This is the angle it subtends with the left half of the frontal plane, and equals the angular distance between the horizontal projection and the left half of the ground line.

Azimuth, elevation and magnitude (H°, V° and M°) are coordinates of the spherical coordinate system. Azimuth ranges from 0° to $\pm$180°. Angles anterior to the frontal plane are considered positive; those posterior are considered negative. Elevation ranges from 0° to $\pm$90°. Angles below the horizontal plane are positive; those above are negative. This is illustrated in the modified polar coordinate chart of Figure 3A. Here the abscissa is the ground line. Vector $\overline{OM}$ has been determined by revolution and is represented here by m^H and M_H. As M_H is obtained by revolution of a horizontal projecting plane and gives us the angle with the horizontal plane, it may be termed the horizontal revolute.

To determine the ventricular gradient in space we need only find its two projections and revolve its horizontal projecting plane orthographically. Any vectorcardiographic lead system is suitable [*11–17*]. Each projection is the result of two components: the frontal, of the transverse (X) and vertical (Y); the horizontal, of the transverse (X) and sagittal (Z). If the areas of the ventricular complex are measured in the component leads, the X, Y, Z coordinates of the



3A 3B

FIG. 3. A, modified polar coordinate chart for determination and recording of spherical coordinates. The values of the components are set off on their respective axes, the projections derived and the revolutions performed. B, spherical coordinates of the gradients of eight normal young men.

gradient are obtained. X is then set off on the abscissa of the chart, Y and Z on the ordinate, the projections derived, and the revolution performed.

RESULTS

The gradients of eight normal young men were determined. The results are shown in Figure 3B. The cube method of electrode placement [*11*] was employed. The areas of PQRST were measured rather than those of QRST for reasons to be discussed. Mean magnitude, azimuth and elevation (M°, H°, and V°) are 47 microvolt-seconds, 16° and 52°, respectively. The pulsed-beam oscilloscope will present accurate amplifications of three simultaneous tracings of fine-line contour. A series of analyses based on such records is planned.

COMMENTS

Wilson, working with discordant T's, established the relationship, $\overline{VG} = \overline{QRS} + \overline{T}$, by empiric means. It has been stated that the ventricular gradient concept is on an insecure theoretic foundation and that its applicability to the normal, with T concordant, is limited [*18*]. This awaits refutation.

A distinction should be made between the waves of activation and regression. The ventricular mass may be considered to be an aggregate of myocardial units. The spatial and temporal sequence in which these are activated is a function of their anatomic relationships to a source of impulse and to the rates of transmission of this impulse from unit to unit in the various regions of the aggregate. When the last unit has been reached, the impulse ceases. After a varying interval the units return to the resting state but the order in which this occurs is no longer dependent upon a spreading impulse. The individual unit receives no signal from its neighbors. It regresses as soon as it is prepared to do so. The duration of the excited state of each unit is a function only of its own metabolic condition and is unrelated to that of the remainder of the aggregate, except to the degree that adjacent units tend to be metabolically similar. In regression, then, there is no self-propagating impulse spreading from a source. There is only an individual emergence of units, independently, from the negative state. The over-all pattern of emergence may resemble a wave or a scatter of contiguous waves, but these differ from those of

activation in their independence of any conducting mechanism.

The gradient is the time-integrated force resulting from differences in duration of the excited state. It is a function of the mean of these differences, an interval of time which for purposes of discussion shall be termed the "time gradient" and annotated by the symbol, TG. If the gradient is called "the net electrical effect of the differences in time course of depolarization and repolarization" [*10*], the time gradient is the net measure of these differences. Such definition of cause and effect allows a more plastic approach to the study of the ventricular complex.

If all the units were metabolically identical the duration of excitation would be a constant, and the gradient equal to zero. The temporal and spatial sequence of regression would duplicate that of activation, and T would be the mirror-image of QRS. It is the localized differences in duration of the excited state that alter T from what may be called its pristine form. However, as the gradient is a function of a mean, it may equal zero even if the local durations of excitement vary, provided the mean difference of the aggregate is zero. In such a case T is not the mirror-image of QRS but the area enclosed by its inscription is equal and opposite to that of QRS; the time-integrated forces of the two phases remain mirror-images. $\overline{T}$ represents the resultant of two vectors, that of its pristine force and that of $\overline{VG}$. Let the former be designated $\overline{T}_0$ (as it is the force that would be generated if there were no differences in duration of excitation). Then $\overline{T}_0 + \overline{VG} \rightarrow \overline{T}$. This is irreversible; the resultant cannot affect its components, T_0 being the mirror-image of $\overline{QRS}$, and $\overline{VG}$ a reflection of those local conditions that influence the duration of excitement. Hence the cordancy of T cannot affect the gradient. However, to determine $\overline{VG}$, the relationship may be considered as an algebraic equation:

$$\overline{T}_0 + \overline{VG} = \overline{T}$$

as

$$\overline{T}_0 = -\overline{QRS}$$

$$-\overline{QRS} + \overline{VG} = \overline{T}$$

or

$$\overline{VG} = \overline{T} + \overline{QRS} \text{ (Wilson's equation)}$$

The theory of the ventricular gradient is predicated on two independent postulates [*9*], the dipole theory of Einthoven [*19–26*] and the hypothesis of limited potential differences of Lewis [*27*]. The first concerns the summation of forces produced in various regions of the myocardium. The heart is considered as a point source of dipole current. The electric activity of its constituent elements affect surface electrodes as though these elements were concentrated at a single point. The electromotive forces generated in the various regions may therefore be summated by the parallelogram law, and the resultant at any instant represented as a single vector, which is grossly termed the instantaneous axis. The second deals with "the relation between the orientation of the electric forces associated with the wave of excitation and the direction in which this wave is moving. . . . at any instant the effective force produced by a muscle fibre is fully determined within that portion of the fibre which is in the process of passing from the resting to the active state. The extent and position of the remaining portions of the fibre are immaterial. It is implied that if two adjacent elements of a muscle fibre differ in their state of activity, one being nearer to or farther from the resting state than the other, there must be an electromotive force across and normal to the plane that separate them" [*9*].

If a muscle element were divided into two portions, A and B, diametrically opposed, as in Figure 4A, and if the duration of excitation of each were one and three seconds, respectively, B would remain in the negative state two seconds longer than A. The net electrical effect of this difference is equal to the integral, or time-integrated force, that would result if B were to remain excited two seconds, and A were not to become activated at all. During this interval a potential difference would exist across the plane separating them which may be termed the boundary-voltage ($\overline{BV}$), a vector quantity directed towards the area with the shorter duration of excitement. Then the integral is $\overline{BV} \times$ two seconds, or $\overline{2BV \text{ seconds}}$, and equals the area enclosed by the tracing. This represents the residue of complete activation and regression of the element and is equivalent to its gradient. The two seconds time difference may also be considered a vector quantity, $\overline{2 \text{ seconds}}$, oriented to the area of shorter duration of excitation. In this case BV is a scalar which when multiplied by $\overline{2 \text{ seconds}}$ results in the same gradient of $\overline{2BV \text{ seconds}}$.

If the element is divided into four portions, A, B, C and D, as in Figure 4B, it contains three

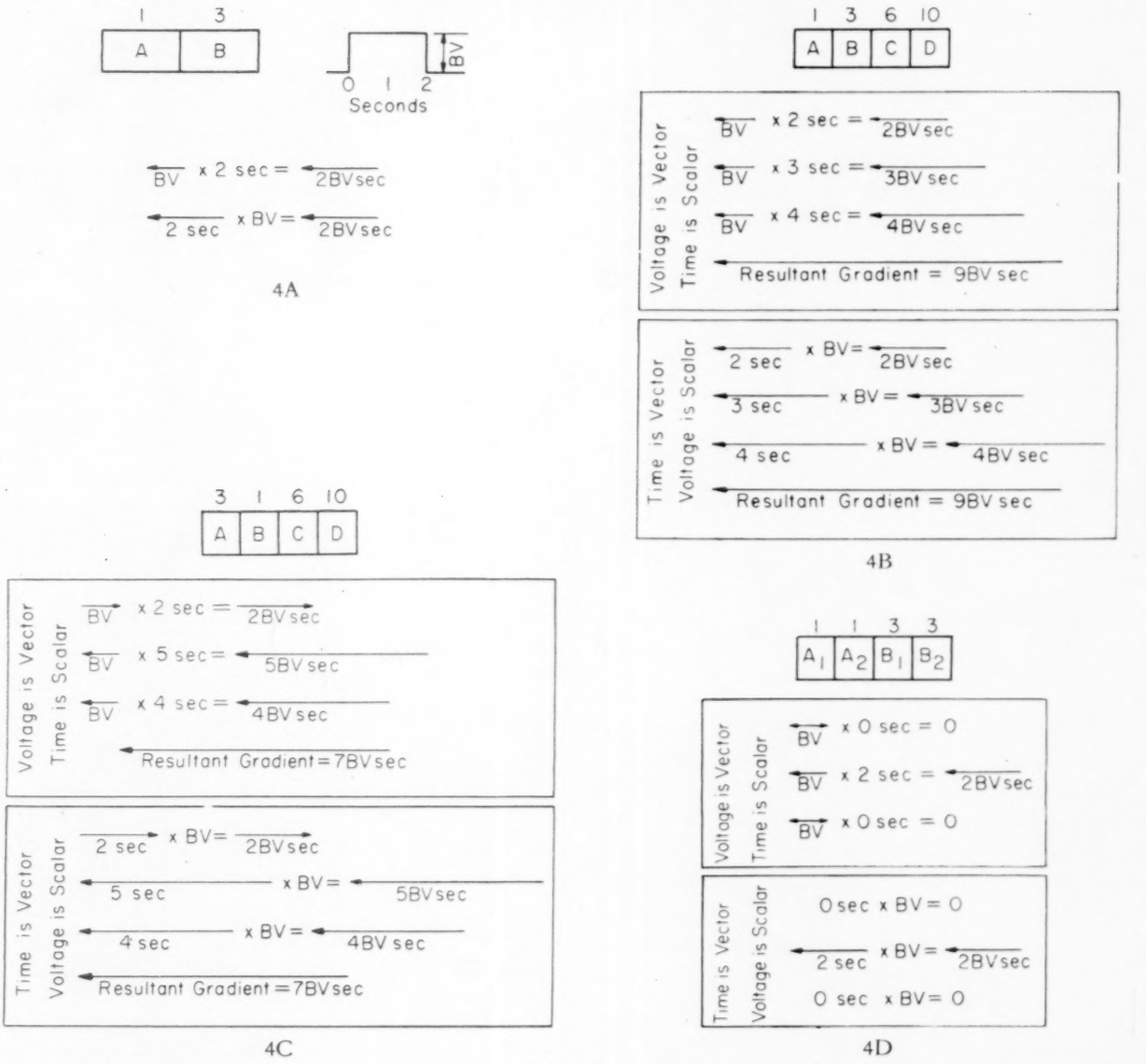


FIG. 4. A through D, the gradient of any strip is equal to (TG)(BV)(n) where either time or voltage is a vector quantity and n equals the number of interfaces.

interfaces. If the durations of excitation of A, B, C and D are one, three, six and ten seconds, respectively, the difference or waiting-period at the A-B interface is two seconds; at the B-C, three seconds; at the C-D, four seconds. The gradient of the strip is then the resultant of the integrals produced by differences across three interfaces, and as each integral is directed towards A, equals 2BV seconds + 3BV seconds + 4BV seconds, or 9BV seconds pointing to A. If the differences in duration across the interfaces are taken as vector quantities, the mean difference in duration of the excited state, or time gradient, across the interfaces is $\frac{2+3+4}{3}$ or 3 seconds, pointing to A. This, when multiplied by the number of interfaces and a scalar BV, equals 9BV seconds, the resultant gradient of the strip.

The same considerations apply if the strip is modified as in Figure 4C. Here the durations of excitement of A and B have been interchanged. As the integral across each interface points to the face with the shorter duration of excitation, the direction of the A-B integral is reversed, and the magnitude of the B-C modified. The time gradi-

ent is then $\frac{5+4-2}{3}$ or $\overline{7/3}$ seconds, oriented to A.

As the element of Figure 4A consists of only two portions, the difference in duration of excitation cannot properly be called a mean. Areas A and B may, however, be subdivided into A_1, A_2 and B_1, B_2, as in Figure 4D. It then contains three interfaces, and as the differences in duration of excitement across A_1-A_2 and B_1-B_2 are zero, and as that across A_2-B_1 is 2 seconds, the mean difference or time gradient of the strip is $\frac{0+0+2}{3}$, or $\overline{2/3}$ seconds. This, when multiplied by the number of interfaces and BV, results in a gradient of $\overline{2BV}$ seconds, which is the same as that of the element without the artificial divisions. The time gradient is therefore a variable and is dependent not only on the metabolic state of the strip but also on the number of segments into which it is arbitrarily divided. When considered as a vector quantity it represents the mean directed difference across the interfaces, and in a given strip the time gradient times the number of interfaces is a constant. The gradient itself of a strip, or of the heart, is not actually a gradient. It is simply a resultant force. The time gradient is, however, a true gradient.

We may say that the gradient of a strip is equal to $(\overline{TG})(BV)(n)$, where n equals the number of interfaces. If we consider an element as a strip divided into two equal segments by a single interface, as that of Figure 4A, its gradient, $\overline{G}$, equals $(\overline{TG})(BV)$. The myocardium is composed of a great number of such elements, the axes of which lie in all directions, and the gradient of the heart is the resultant in space of these elementary gradients. $\overline{VG}$ is the genetic product solely of the coefficients $\overline{TG}$ and BV, both of which are expressions of intrinsic qualities of the myocardium. Its magnitude and orientation are fixed by these alone and are influenced by no other force. The courses of accession and regression affect the gradient no more than waves on a pool affect the composition of the water, but the values of the forces generated serve to determine $\overline{VG}$ when substituted in the equation: $\overline{VG} = (\overline{QRS} + \overline{T})$.

It might seem from this discussion that the duration of excitation of an inner zone, e.g., C of Figure 4C, exerts as much influence on the gradient, and therefore on the T wave, as that of an outer zone such as D. Upon close scrutiny of Figure 4C, however, a curious truism becomes apparent. The gradient, $\overline{7BV}$ seconds, is equal to that which would exist if B and C were excluded from the strip and A and D were contiguous. Moreover, if the latter remain three and ten seconds, respectively, any alteration of B and C is without effect on the resultant gradient. This, of course, is corollary to the thesis stated by Wilson [*8*] and amplified by Bayley [*28*] that the duration of the excited state in muscle layers between subsurface layers of the myocardium is immaterial.

By application of the equation, $(\overline{TG})(BV) = \overline{VG}$, it is possible to impose a gradient on a hypothetic muscle strip. Let us examine such an isolated strip (Fig. 5A) immersed in a homogeneous conducting medium. It consists of two halves, each 1 cm. long, WX and YZ. Two electrodes, equidistant from the center, are remotely placed on its axis and connected to a galvanometer. The recording paper of the latter moves in a direction perpendicular to the strip above Z, and the polarity of the electrodes is such that an upward deflection is recorded when the vector representing the electromotive force of the strip points to Z. The deflection of the tracing at all times will be concordant with the vector and of an amplitude proportional to its magnitude. The electric nature of the strip is such that a deflection representing 1 volt is recorded when an excited zone is contiguous to a resting zone along a plane, or boundary, perpendicular to the axis of the strip. This is so regardless of the position of the boundary as, in keeping with the dipole theory, the entire strip may be considered to be concentrated at a single point midway between the electrodes. BV of the strip is therefore equal to 1 volt. Both WX and YZ have the property of remaining in the excited state for three seconds. This is annotated as: E = 3, for both WX and YZ. The gradient of the strip is therefore equal to zero. A wave of excitation travels at an assumed rate of 1 cm. per second.

If a stimulus is applied in the region of W, activation starts immediately. In the figure, black represents the excited state (negativity); white, the resting state (relative positivity). The condition of the strip at the end of each second is illustrated. The table is a log of events occurring in the various regions at the end of each second and during the interims. The sequence of events is described in the legend. It is worth

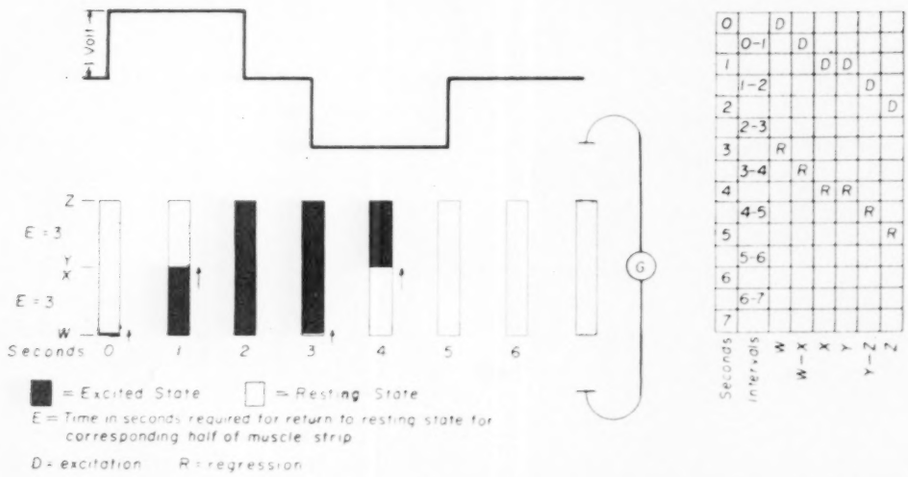


FIG. 5A. The duration of excitement, E, is three seconds throughout. The gradient is therefore zero. When a stimulus is applied at W, a wave of excitation passes to Z. As the strip is of constant cross-section, and as the electrodes are to be considered remotely situated, a deflection of 1 volt is recorded regardless of the position of the wave front. At 2, the entire strip is in the negative state, and the potential difference between the electrodes is zero. The tracing therefore returns to the baseline. At 3, W has remained in the excited state for three seconds, and regression therefore begins. The area of QRS and T equals zero. The table notes the times of activation and regression of the various regions.

noting that the progression of white (regression) from 3 to 5 is not of the same nature as that of black (activation) in that there is no conduction mechanism in the former. The re-emergence of whiteness at a uniform rate is a consequence of the inherent disposition of all parts of the strip to remain excited for the same length of time, the progression of blackness being therefore duplicated. It is seen that the second phase is the mirror-image of the first. If the latter be taken as QRS, the former is $-$QRS or T_0.

Let us assume that the metabolic state of region YZ has been altered so that it is now disposed to remain excited for seven seconds, i.e., $E_{YZ} = 7$. As E_{WX} remains equal to 3, we have imposed a time gradient of four seconds, and as BV equals 1 volt, the gradient ($\overline{G}$) is 4 volt-seconds. The results are shown in Figure 5B. T has been altered by the area, lmno, which represents the gradient and encloses an area of 4-volt-seconds oriented towards WX, the region that remains in the excited state for the shorter length of time. The area of T equals that of T_0 plus lmno, and the area of lmnq equals that of QRS plus T.

In Figure 5C, the gradient of the strip is as in 5B but the stimulus is applied at Z. ($\overline{QRS} + \overline{T}$) is still 4-volt-seconds and points again to WX. It is seen that, regardless of the course of excitation and the cordancy of T, $\overline{G}$ points to the area that remains in the excited state for the shorter interval, and its magnitude in volt-seconds is equal to the vector sum of $\overline{QRS}$ and $\overline{T}$.

To test the effect of an inconstant rate of activation let us examine the strip in Figure 5D. Here the gradient is unchanged but the nature of the segment, WM, has been altered so that the speed of the excitation front is slowed to 0.5 cm. per second as it traverses this area. The stimulus is applied at W. The area of QRS is increased by abcd, but the magnitude and orientation of the gradient (lmno) remain unaffected. $\overline{T}$ is again the resultant of $\overline{T}_0$ and $\overline{G}$ and differs from that of Figure 5B by the secondary T wave change, efgh, which is equal to abcd.

In Figure 5E, the stimulus has been applied at Z and there is again slowing of the wave of excitation at WM. The gradient, lmno, is still 4 volt-seconds oriented to WX and equals ($\overline{QRS} + \overline{T}$). It may be inferred that inconstancy of rate of depolarization does not affect $\overline{G}$. This is to be expected as the gradient has been arbitrarily imposed on this hypothetic strip, as it is on the ventricles by intrinsic qualities of the muscle.

If the strips of Figure 4B and C be subjected to activation from either end at varying rates, the same results are obtained.

We have been working with a single element, on the axis of which the forces of excitation, regression and the gradient are superimposed. The electrical events of the heart, however, are

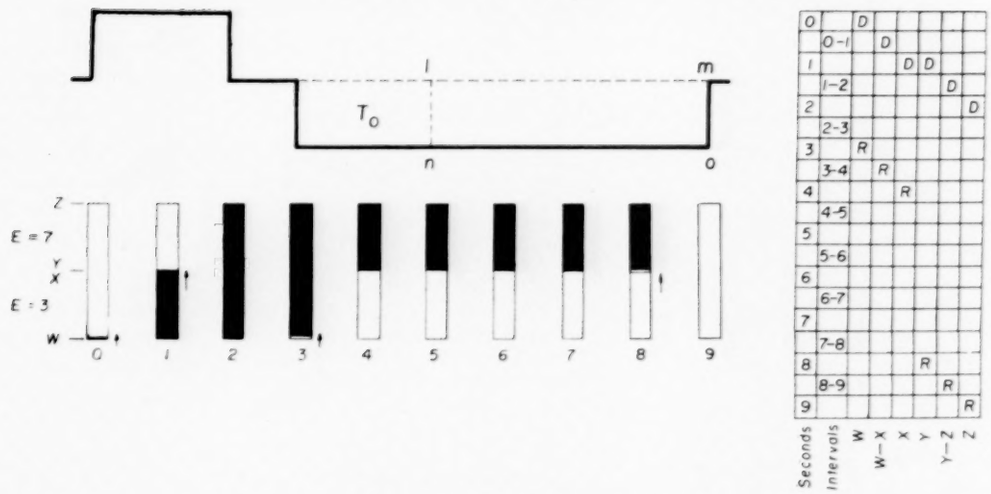


FIG. 5B. E_{YZ} = seven seconds. E_{WX} = three seconds. $\overline{TG}$ therefore equals four seconds. As BV = 1 volt, $\overline{G}$ = 4 volt-seconds. W, WX and X remain excited for three seconds; Y, YZ and Z, for seven seconds. T has been altered by the area, lmno, which represents 1 volt oriented towards W for four seconds and is a measure of the gradient, $\overline{G}$. The area of QRS + T = 4 volt-seconds, and the area of T equals that of T_0 plus lmno.

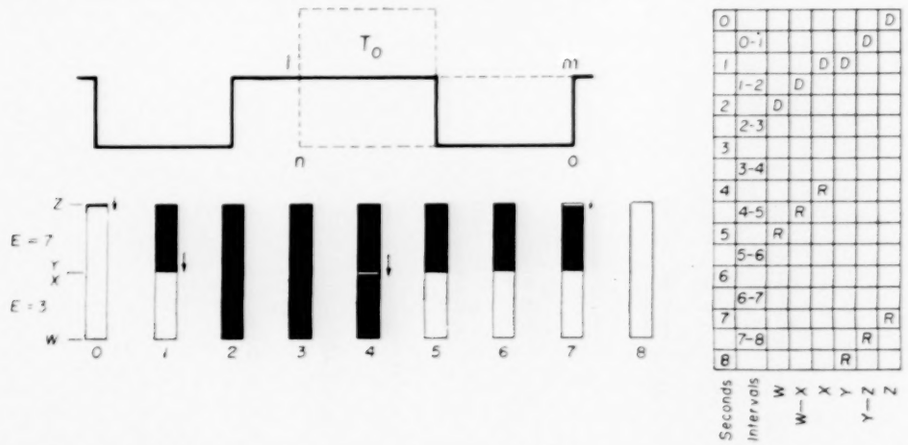


FIG. 5C. The strip is unchanged, but the stimulus is applied at Z. $\overline{T}$ is equal to $\overline{T}_0$ + lmno. The latter is still 4 volt-seconds oriented to WX and equals $\overline{QRS} + \overline{T}$.

spatial. The constituent elements lie in all directions, and the vector of its gradient is the resultant in space of the elementary gradients. In Figure 6A, four muscle spindles are depicted, each representing a major contributing area of the myocardium. For purposes of exposition it shall be assumed that these are of constant cross-section throughout their length, i.e., rods. The arrows indicate the direction of excitation. RV (right ventricle) and Sm (septum) are activated anterosuperiorly to the right; LV (left ventricle), posteroinferiorly to the left; Ax (apex), anteroinferiorly to the left. They comprise a hypothetic heart. The orthographic projections of these spindles are shown in Figure 6B, and their properties in Table 1. The listed $\overline{TG}$, BV and $\overline{G}$ values are projections of the spatial magnitudes and are oriented along the axes of the projections of their respective spindles. In Figure 6C, the projections of the resultant gradient are obtained by vectorial summation of the constituent gradient vectors. The X, Y, Z components, as taken from its projections, are 1.0 volt-seconds, −1.7 volt-seconds and 3.3 volt-seconds respectively.

Excitation starts in Sm at 0 seconds and in RV, LV and Ax at one second. The state of the myocardium and the instantaneous resultant vector at the end of each second are shown by projection in Figure 6D. The XYZ components

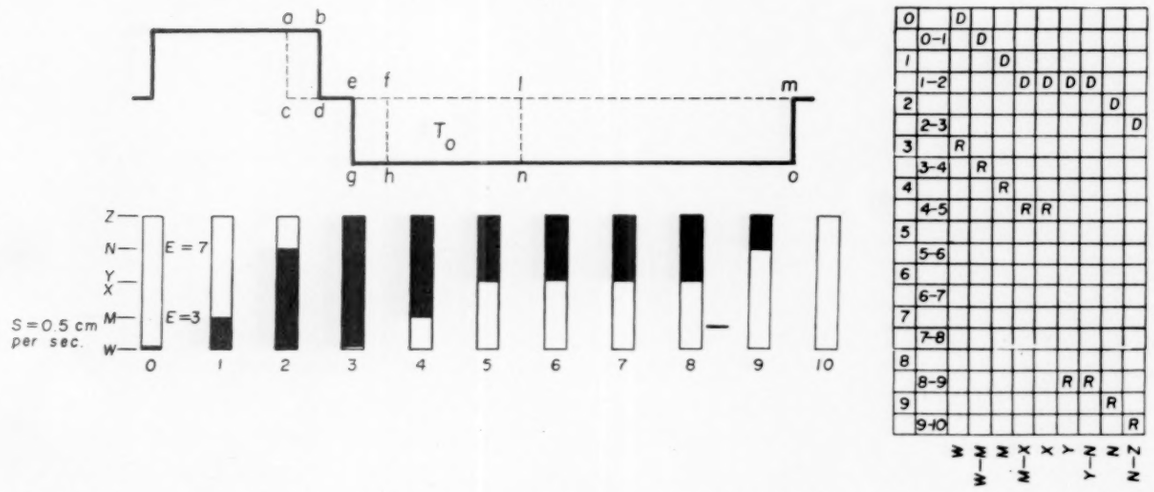


FIG. 5D. The time gradient is unchanged, but the nature of segment WM has been altered so that the speed of activation is slowed to 0.5 cm. per second as it traverses this area. The stimulus is applied at W. The first phase is altered by the area, abcd. $\overline{T}_0$ has been modified by efgh, which is equal to abcd. The magnitude and orientation of $\overline{G}$ (lmno) have been unaffected by the inconstant rate of activation. $\overline{T}$ is again the sum of $\overline{T}_0$ and $\overline{G}$ differs from that of Figure 5B by the secondary T wave change, efgh.

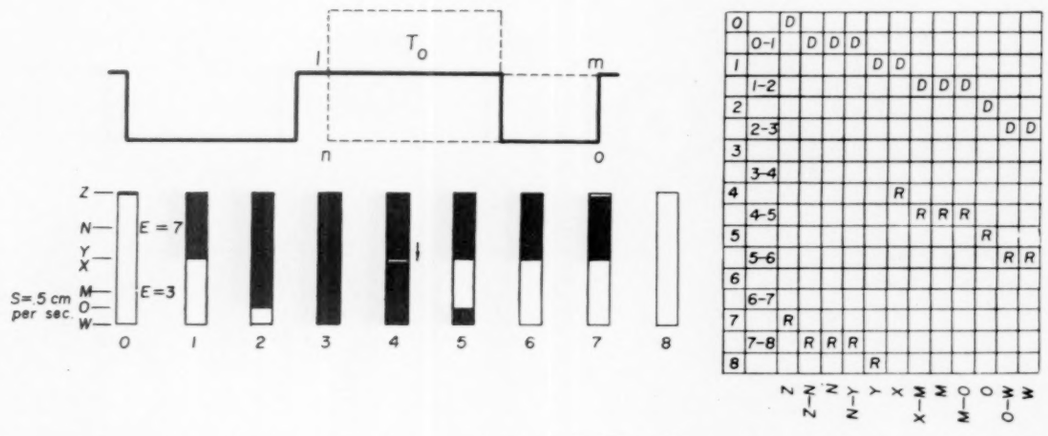
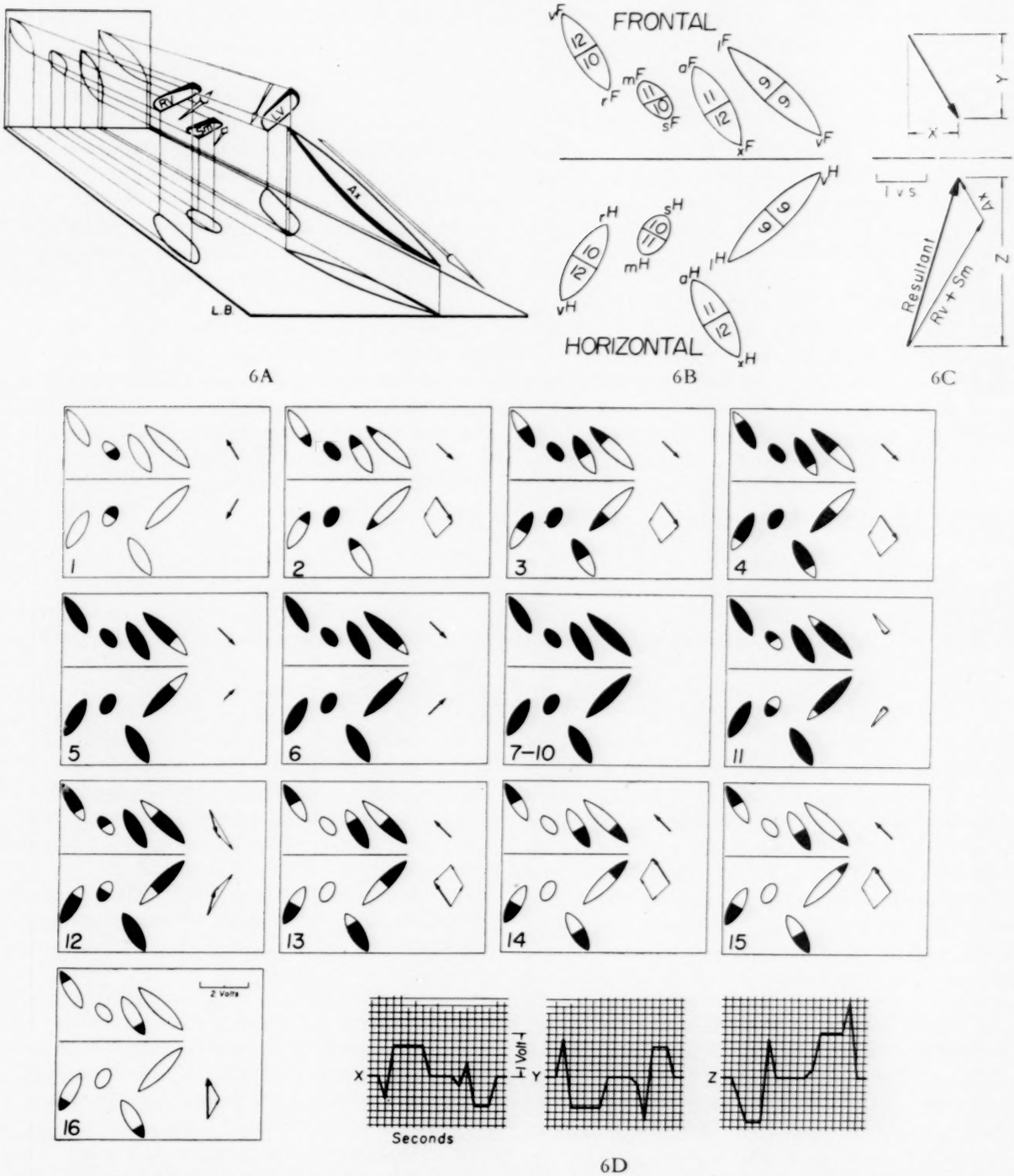


FIG. 5E. The strip is unchanged, but the stimulus is applied at Z. $\overline{QRS} + \overline{T}$ is 4 volt-seconds and equals lmno, the gradient. $\overline{T}$ again equals lmno + $\overline{T}_0$.

TABLE I

PROPERTIES OF THE SPINDLES OF FIGURE 6

Spindle	$\overline{TG}$ (seconds)	Horizontal Projection of BV (volts)	Horizontal Projection of Gradient (volt-seconds) ($\overline{G} = \overline{TG} \times BV$)	Frontal Projection of BV (volts)	Frontal Projection of Gradient (volt-seconds) ($\overline{G} = \overline{TG} \times BV$)	Gradient Oriented Toward	Activation Starts at (seconds)
RV	2	1	2	1	2	R	1
Sm	1	1	1	1	1	S	0
Ax	1	1	1	1	1	A	1
LV	0	1	0	1	0	..	1



6A 6B 6C

6D

FIG. 6. A, hypothetic heart. Four muscle spindles are shown, each representing a major contributing area of the ventricular mass. The arrows indicate the direction of excitation. RV (right ventricle) and Sm (septum) activate anterosuperiorly to the right; LV (left ventricle), posteroinferiorly to the left; Ax (apex), anteroinferiorly to the left. B, orthographic projections of the spindles. The durations of excitement are indicated. Their properties are listed in Table 1. $\overline{TG}$ of RV, for example, is two seconds and the horizontal projection of its BV is 1 volt. The horizontal projection of the gradient of RV = 2 × 1 = 2 volt-seconds oriented to R. C, the projections of the gradients of the spindles are vectorially summated, and the XYZ components of the resultant obtained. X = 1.0 volt-seconds; Y = −1.7 volt-seconds; Z = 3.3 volt-seconds. Note that the gradient of LV is zero. D, excitation starts in Sm at zero seconds and in RV, Ax and LV at one second. The condition of the spindles at one second intervals is shown by projection, and the projections of the instantaneous resultants obtained by vectorial summation. The XYZ components have been derived from these and integrated to form the linear tracings at the bottom of the figure. The net areas of X, Y and Z are 1.0 volt-seconds, −1.7 volt-seconds and 3.3 volt-seconds, respectively, as anticipated in Figure 6C.

of each resultant have been derived and successively integrated to trace the curves shown in this figure. By areal measurement, X = 1.0 volt-second, Y = −1.7 volt-seconds and Z = 3.3 volt-seconds, as anticipated. The spatial gradient established by $\overline{TG} \times BV$, terms descriptive of the muscle itself, has been determined by measurement of waves passing through the muscle, QRS and T. Construction for magnitude and orientation is left to the reader.

As the gradient of an element is the product of two coefficients, it is accordingly modified by alteration of these quantities. $\overline{TG}$, the time gradient, is dependent on those factors that govern local duration of excitation. BV, the boundary voltage, is a measure of the potential difference across a boundary dividing resting from activated muscle and is a function of the area of this boundary. Thus, if the element of Figure 4A is taken as cylindric in form, the area of the interface between A and B is πr^2, and the boundary voltage is therefore in proportion to the square of the radius. In Figure 6A the ventricular musculature is represented by four elements. It is evident that localized hypertrophy, to the extent that it increases the radius of one of these elements, will result in an alteration of both magnitude and orientation of the resultant gradient. Generalized hypertrophy, by equally modifying all the elements, will effect a change only in its magnitude.

Irritability also affects the gradient. It determines the degree of electrical response to an impulse or the intensity of excitation. Augmented irritability is accompanied by an increase in voltage across the interface separating resting from excited muscle. Local and generalized changes in irritability therefore modify the gradient, the former being accompanied by alteration in both magnitude and orientation, the latter, in magnitude alone. In a given heart, therefore, two independent variables, local irritabilities and local durations of excitation, may effect a change in gradient between successive complexes.

The five intramyocardial factors that determine the size and configuration of the ventricular complex may now be tabulated:

$$\left.\begin{array}{l} \text{QRS} \left\{\begin{array}{l} \text{Conduction pathway} \\ \text{Velocities of impulse} \\ \overline{\text{VG}} \left\{\begin{array}{l} \text{Size and distribution of muscle mass} \\ \text{Local irritabilities} \\ \text{Local durations of excitement} \end{array}\right. \end{array}\right. \\ \text{T} \end{array}\right\}$$

T is dependent on all five; QRS, on the first four, $\overline{VG}$ on the last three. As a change in the first four will equally affect both QRS and T, it follows that a primary T wave change results only from alteration of the last factor. An alteration in gradient, on the other hand, may be brought about by modification of any of the last three.

The role of the RS-T segment in gradient analysis has received little consideration in the literature. After inscription of QRS, the entire ventricular mass is in a negative state. The level of RS-T, then, regardless of its height, expresses a potential difference of zero. T is inscribed as the mass returns to the resting state. Should unfavorable metabolic conditions prolong the duration of excitation beyond the physiologic in a subsurface group of fibres, this will be reflected as a primary T wave change, indicative of an altered gradient. As local metabolic conditions worsen, eventually the persistence of negativity will outlast the complete regression of the remainder of the myocardium. A patch of muscle will then remain excited after T is inscribed, and the potential difference between it and the normal fibres that have returned to rest is registered as a deviation of the T-P interval from the level of RS-T. As the former is customarily taken as the baseline, this difference in levels is read as an RS-T elevation or depression. Should the refractory area or any portion of it become deactivated after the onset of the succeeding P, it will return to the excited state during inscription of the subsequent QRS, so that the difference in levels of T-P and RS-T remains fixed as though an area of injury were perpetuated and constantly generating a current of injury.

As an RS-T deviation of the injury type signifies a region that has remained excited at least up to the onset of the succeeding P and for an unknown period thereafter, it is impossible in its presence to determine the gradient of the entire myocardium. Should localized negativity persist for weeks, for example, the gradient could obviously not be found by measuring the area of a single complex. Moreover any need for doing so is obviated as the RS-T deviation is sufficient evidence of an alteration in the state of the muscle. However, RS-T deviation does not always signify damage. In most instances it is caused by an overlap of accession and regression, i.e., the inscription of T begins before that of QRS has ended. An experienced electro-

cardiographer can usually but not always distinguish these two types of RS-T changes and there is the possibility of a combined genesis, both damage and overlap contributing to the deviation. Here determination of $\overline{VG}$ may prove useful if a previous record without deviation or a table of norms (as yet unprepared) is available for comparison. If overlap is the sole cause of the RS-T deviation, $\overline{VG}$ should be similar to that of the control as the area enclosed by RS-T represents the resultant of the overlapped portions of QRS and T. If injury plays a role, erroneous coordinates will be assigned to an indeterminable force, and the resultant vector will differ from the control.

The small series presented herein was obtained from photographic tracings at normal standardization and triple speed, enlarged ten times. At this magnification, differences in levels of the T-P interval, P-R interval and RS-T segment are usually apparent. These may result from the following:

Intramyocardial Factors. (1) Persistence of an area of negativity. It must be assumed that, in these normal subjects, all activated areas have returned to the resting state at the completion of T. (2) Onset of regression before completion of QRS. This usually occurs and is manifested as a slow development of T from the terminal limb of QRS or by frank overlap. (3) Regression of the atria during the P-R interval and ventricular complex. Berkun and his associates [*29*] have demonstrated that the T of P, Tp is approximately equal and opposite to P, i.e., that the auricular gradient is close to zero. If T-P is taken as the isoelectric level and the area of PQRST measured algebraically in one sweep of the planimeter, the aberrative effects of both overlap and Tp are obviated. P and Tp negate each other and the area of RS-T included in the sweep represents the resultant of the overlapped portions of QRS and T.

Extramyocardial Factors. (1) Respiratory variations. (2) Machine-induced variations. These may be minimized by a careful choice of complexes. Their effect on areal values is proportionate to the paper speed. The usual speed of 25 mm. per second is therefore preferable to a higher speed.

Certain factors affect the values obtained. (1) Chest wall configuration and conditions within the chest, (2) heterogeneity of conducting tissue, (3) distance of electrodes from the dipole-center, (4) validity of the cube; do the electrodes truly bear an isometric relationship to the dipole-center; and (5) rotation of the heart during inscription of the ventricular complex.

These influences are inherent in any system of surface electrode recording. Their consideration is outside the scope of this paper.

SUMMARY

1. A simple, geometric method of determining the spherical coordinates of the ventricular gradient is presented.

2. Electrocardiograms of eight normal subjects are thus analyzed, using the cube method of electrode placement. Mean magnitude, azimuth and elevation are found to be 47 microvolt-seconds, 16° and 52°, respectively.

3. The theory of the gradient is discussed.

4. The role of the RS-T segment is elucidated and a method of evaluating its significance is suggested.

5. Gradients are imposed on hypothetic muscle strips by means of a suitable equation, and the effects of change in rate and direction of activation on the form and area of the ventricular complex are demonstrated. It is concluded that these do not influence net area.

6. A block diagram of an instrument for instantaneous determination of the gradient is included. (See addendum.)

ADDENDUM

If we re-examine Figure 2B, we find that TL and $<H$ are the polar coordinates of OM in its revolved position on the frontal plane. $<H$ could be derived from the equation:

$$<H = \tan^{-1}\frac{M_H m_H}{Om_H}$$

as it has not changed during revolution of the horizontal projecting plane. $M_H m_H$ and Om_H of Figure 2B are respectively equal to Y and Om_H of Figure 2C. In Figure 2C, therefore

$$<H = \tan^{-1}\frac{Y}{Om_H}$$

but $$Om_H = Om^H$$

and $$Om^H = \sqrt{X^2 + Z^2}$$

therefore $$<H = \tan^{-1}\frac{Y}{\sqrt{X^2 + Z^2}}$$

TL, the magnitude, is equal to $\sqrt{Y^2 + (Om_H)^2}$, and therefore $= \sqrt{X^2 + Y^2 + Z^2}$. This is Pythagoras's equation.

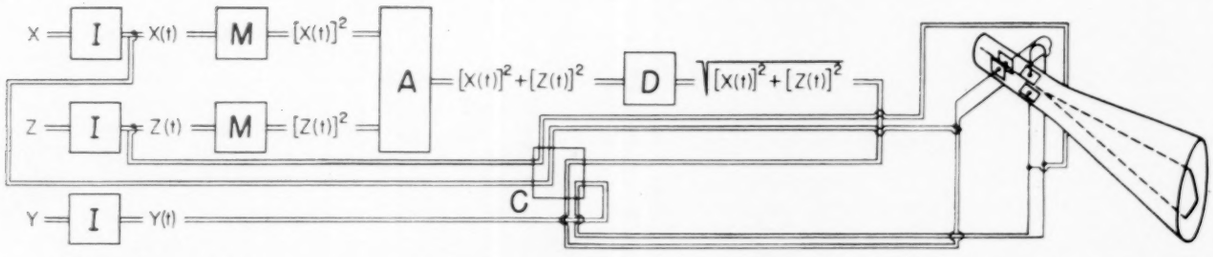
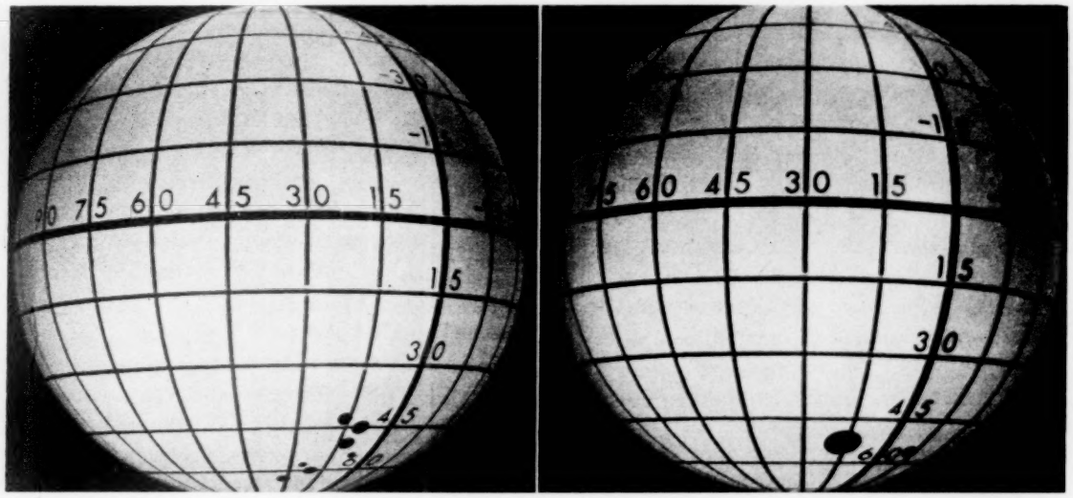


FIG. 7. Pulsed-beam circuit for instantaneous recording of spherical coordinates of the ventricular gradient. X(t), Y(t) and Z(t) are the Cartesian coordinates of the integral of the electromotive forces generated during excitation and regression. I is integrator; M, multiplier; A, adder; D, for derivation of square root. C is electronic switch pulsing beam between the circuits of the horizontal projection and the horizontal revolute of the gradient.



8A 8B

FIG. 8. A, spherical representation of eight gradients. B, mean $\overline{QRS}$ and $\overline{VG}$ vectors. $\overline{VG}$ subtends an angle of 17° with $\overline{QRS}$ and bears N4°, W28°, from $\overline{QRS}$.

The spatial coordinates can be obtained instantaneously by instrumental means. The Cartesian coordinates of the horizontal revolute, M_H, are Y and $\sqrt{X^2 + Z^2}$. If the PQRST areas of the three components are measured by an area integrator [*30*], the X, Y, Z components of the gradient are electronically obtained. $\sqrt{X^2 + Z^2}$ is then led to the horizontal terminals of the oscilloscope and Y to the vertical. The horizontal revolute will be flashed on the screen. The ideal is an oscilloscope with an electronic switch giving simultaneous H revolute (magnitude and elevation) and H projection (azimuth). The block diagram of such an instrument is given in Figure 7.

If we picture the null-point as lying at the center of a sphere [*30*], a vector radiating outwards will pierce its surface at a point where longitude and latitude are respectively equal to the azimuth and elevation of the vector. The eight gradients determined are so illustrated in Figure 8A. Here magnitude is represented by the radius of the spots.

In normal subjects $\overline{VG}$ and $\overline{QRS}$ are roughly concordant. The $\overline{QRS}$ axis may therefore be used as a reference coordinate for that of $\overline{VG}$, and the $\overline{QRS}$-$\overline{VG}$ angle determined by positioning these vectors on the surface of the sphere and measuring the arc distance between them with a great circle or a pair of dividers. The $\overline{QRS}$-$\overline{VG}$ angle ranges from 4° to 32° with a mean of 17°. (Fig. 8B.) The bearing of the mean $\overline{VG}$ axis from that of the mean $\overline{QRS}$ axis is N4°, W28°. The gradients lie within a solid angle of 21°.

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Studies of Neurohypophyseal Function in Man*

Diabetes Insipidus and Psychogenic Polydipsia

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MARKED polydipsia and polyuria in the absence of glycosuria are rarely encountered in clinical practice. Once diabetes mellitus has been excluded, the differential diagnosis generally rests between diabetes insipidus and psychogenic polydipsia. Most cases of diabetes insipidus are caused by a decrease or absence of antidiuretic hormone (ADH) secretion from the neurohypophysis [*1*], although rare instances of a congenital refractoriness of the renal tubules to ADH have been described [*2*]. Psychogenic polydipsia is usually observed in emotionally disturbed persons, and is believed to represent a defect in thirst control rather than in ADH secretion.

The neurohormonal unit responsible for ADH production and secretion, the neurohypophysis [*3*], includes the supraoptic and paraventricular nuclei, the supraoptico-hypophyseal tract, and the posterior pituitary gland. Although older concepts attributed the function of ADH production to the posterior pituitary, the studies of Scharrer [*4,5*] and Bargmann [*6,7*] suggest that ADH is produced by a process of neurosecretion in the neurones of the hypothalamic nuclei in question, is then transported distally along the axones of the tract, and stored in end-plates about vascular channels in the posterior pituitary or secreted directly into blood vessels of this region.

There are two known routes of stimulation of the neurohypophysis resulting in ADH secretion. The first is probably through neuronal pathways leading to activation of the hypothalamic nuclei by noxious agents and psychic stress [*8,9*], and by drugs such as acetylcholine ("nicotinic action") [*10*] or nicotine [*11*]. The other route is by way of stimulation of an "osmoreceptor" somewhere within the distribution of the internal carotid arteries which invokes ADH secretion from the neurohypophysis by responding to alterations in the osmotic pressure of the blood [*8*]. Appreciation of these physiologic mechanisms permits a better understanding of the specific functional impairment which may be responsible for disorders of hydration implicating this endocrine system.

The present report deals with a study of a group of patients with polyuria to whom nicotine was administered to test the integrity of the hypothalamic nuclei, hypertonic saline solution to assess the function of the osmoreceptor, and pitressin® to evaluate the response of the renal tubules to ADH. Serial intravenous administration of each substance in turn under constant water-loading conditions has allowed a fairly complete clinical evaluation within a four- to six-hour period, with minimal inconvenience and discomfort to the patient. The results of this study have permitted the separation of patients into three categories: (1) true diabetes insipidus with severe insufficiency or complete loss of neurohypophyseal function, (2) a diabetes insipidus-like syndrome in patients with posterior pituitary damage or vascular disease characterized by a loss of osmoreceptor function but with evidence suggesting preservation of hypothalamic centers for ADH production, and (3) psychogenic polydipsia with a normal osmoreceptor response but some abnormality in the hypothalamic response to nicotine which may be related to adrenal steroid excess. Lewis and

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Chalmers [*12*] have previously described one patient who satisfied the diagnosis of osmoreceptor failure as herein described, and they commented on the possible nature of the defect. The disordered response to nicotine in patients with polydipsia has not been previously reported.

MATERIAL AND METHODS

Pitressin tannate in oil (PTO) therapy was withdrawn from patients requiring its use, and extreme polyuria was controlled with the administration of 5 units of aqueous pitressin subcutaneously every four to six hours. This short-acting preparation was discontinued at least twelve hours before the study to insure a state of polyuria. Free access to water was allowed since prior dehydration was found to be unnecessary and extremely discomforting. A small breakfast of toast and jelly and 100 to 200 ml. orange juice was allowed about 7:00 A.M. on the day of the study. Between 8:00 and 9:00 A.M. the patients drank 20 ml. water per kg. of body weight within a fifteen- to thirty-minute period. Urine was collected by indwelling catheter at fifteen- to thirty-minute intervals with the subjects in the recumbent position. A constant state of hydration and of diuresis was maintained by having the subjects drink a volume of water equal to the urine flow for each collection period. In some instances 5 per cent dextrose in water was injected intravenously after the oral water load to maintain hydration. When peak diuresis, usually exceeding 5 ml. per minute, occurred, a nicotine salicylate solution* was injected intravenously over a three- to five-minute period; usually 1 mg. nicotine base was given to non-smokers, and 3 mg. to smokers, as recommended by Cates and Garrod [*21*]. When urine flow returned to preinjection levels, 15 to 25 milliunits aqueous pitressin† was administered intravenously as a single injection or as a one-hour infusion in 5 per cent dextrose in water. At the termination of antidiuresis a 3 per cent sodium chloride solution (10 ml./kg.) was infused rapidly over a thirty- to forty-five-minute period. Studies were terminated thirty to sixty minutes after infusion of the saline solution. Water administration was discontinued usually with the pitressin injection to allow a decrease in the fluid balance in order to minimize dilution of the hypertonic saline solution. Blood specimens were obtained from an antecubial vein prior to hydration, at the peak of water diuresis, and at the end of each infusion. Creatinine (cr) was measured by the method of Bosnes and Taussky [*13*], chloride (Cl) by the method of Schales and Schales [*14*], and sodium and potassium concentrations in urine and plasma were determined by flame photometry using lithium as an internal standard. Total solute concentrations of urine and plasma were measured by freezing point depression using a Thermistor 1 B resistance probe and a Wheatstone bridge osmometer.* Changes in glomerular filtration rate were estimated by the endogenous creatinine clearance in ml. per minute (C_{cr}), or creatinine excretion in mg. per minute ($U_{cr}V$). The following calculations were employed [*15*]:

$$\text{Osmolar clearance in ml./min. } (C_{osm}) = \frac{U_{osm}}{P_{osm}} V$$

U_{osm} = total solute concentration of urine in milliosmoles (mOsm)/L.
P_{osm} = total solute concentration of plasma in mOsm/L.
V = urine flow in ml./min.

$$\text{Free water clearance in ml./min. } (C_{H_2O}) = V - C_{osm}$$

C_{H_2O} is positive during water diuresis, and becomes negative when urine is hyperosmolal to plasma. A sharp decrease in C_{H_2O} during water diuresis indicates an action of ADH on water reabsorption by the renal tubules. This index is of greatest use in demonstrating the effect of ADH on water reabsorption during the osmotic diuresis which frequently occurs with administration of three per cent saline solution. In this instance a large increase in osmolar clearance could actually produce a rise in urine flow although increased water reabsorption with a decline in C_{H_2O} may have occurred. Other indexes of antidiuresis were changes in the osmotic U/P ratio and in urinary concentrations of creatinine (U_{cr}), and chloride (U_{Cl}). The rate of excretion of Cl (in mEq./min.) equalled $U_{Cl}V$.

CASE REPORTS

CASE 1. (Fig. 1.) A thirty-two year old white woman had diabetes insipidus of thirteen years' duration which was associated with Hand-Schüller-Christian disease. Five units of PTO at four-day intervals were required to control polyuria. Amenorrhea had been present for the preceding two years; this was associated with low urinary follicle-stimulating hormone (FSH) levels. Thyroid and adrenal studies were normal. The patient was a non-smoker, and had polyuria of approximately 7 L. per day when not receiving pitressin therapy. No antidiuresis was

* Obtained from Fine Organics, Inc., New York, New York. Nicotine solution prepared by dissolving 185 mg. nicotine salicylate in 500 ml. 5 per cent dextrose in water, acidifying with one drop of 50 per cent sulfuric acid, and sterilizing in an autoclave. Five ml. of solution contain 1 mg. nicotine base.

† Supplied through the courtesy of Dr. E. C. von der Heide, Parke Davis Co., Detroit, Michigan.

* Fiske Associates, Danvers, Massachusetts. The authors wish to express their gratitude to Doctors J. P. Merrill and J. T. Finkenstaedt for the use of their apparatus.

observed with either intravenously administered nicotine or hypertonic saline solution, although severe vertigo and vomiting occurred with 2 mg. of nicotine. A normal response to pitressin was observed. C_{osm} increased slightly with hypertonic saline infusion.

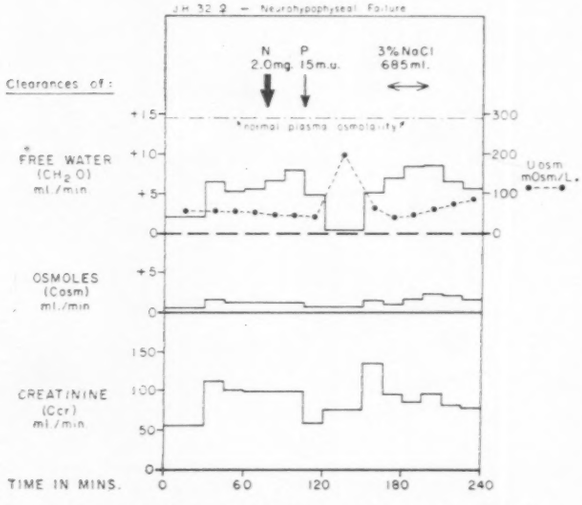

FIG. 1. Case I. Effect of nicotine and hypertonic saline on ADH secretion. In this and subsequent figures, the following abbreviations are employed: N = nicotine (mg.), P = pitressin (milliunits), U_{osm} = urinary total solute concentration, (mOsm/L.), U_{Cl} = urinary chloride concentration (mEq./L.), U_{cr} = urinary creatinine concentration (mg./100 ml.).

CASE II. (Fig. 2.) A sixty-five year old white man, who was an occasional cigar smoker, noted the sudden onset of polydipsia and polyuria a few months before this study. These symptoms were controlled by intranasal insufflation of posterior pituitary powder. Mild diabetes mellitus was discovered and controlled adequately with the administration of 20 units of NPH insulin daily; however, polyuria of 6 to 7 L. per day persisted in the absence of glycosuria. No antidiuresis occurred after two injections of nicotine (each 1 mg.) administered at forty-five-minute intervals, or with hypertonic saline solution. Vertigo and nausea without vomiting occurred with nicotine administration. Fifteen milliunits pitressin in 5 per cent dextrose in water administered intravenously over a sixty-minute period produced a marked antidiuresis and a rise in U_{cr} and U_{Cl}. An increase in chloride excretion occurred when hypertonic saline solution was administered.

CASE III. (Fig. 3.) Polydipsia and polyuria developed in a forty-five year old white salesman four months prior to this study. He had never received pitressin therapy. The urine volume average was 6 to 7 L. per day. A six-hour period of dehydration resulted in a 1,410 ml. urine loss, accompanied by extreme thirst and dryness of the mouth. The urine total solute concentration rose to 257 mOsm/L. in the specimen collected between the fourth and sixth hour, with serum osmolality measured at 290 mOsm/L. These results denoted inability of the kidney to concentrate the urine beyond the concentration of the plasma when dehydration was produced, suggesting moderate ADH deficiency. The occurrence of vertigo, hyperpnea and nausea prompted discontinuance of the nicotine infusion when 2 mg. had been administered. A moderate decline in C_{H_2O} and in urine flow occurred. A rise in osmotic U/P from 0.25 to

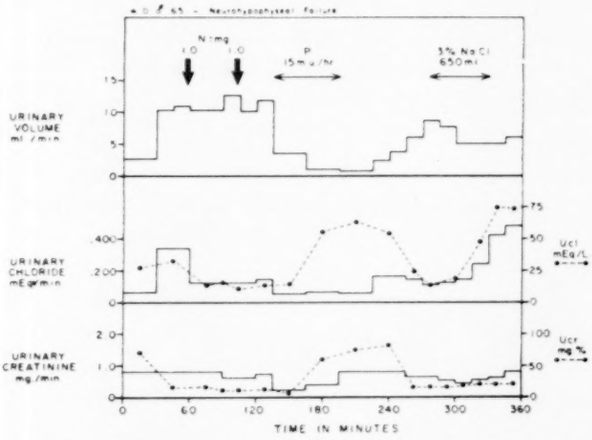

FIG. 2. Case II. Effect of nicotine and hypertonic saline on ADH secretion.

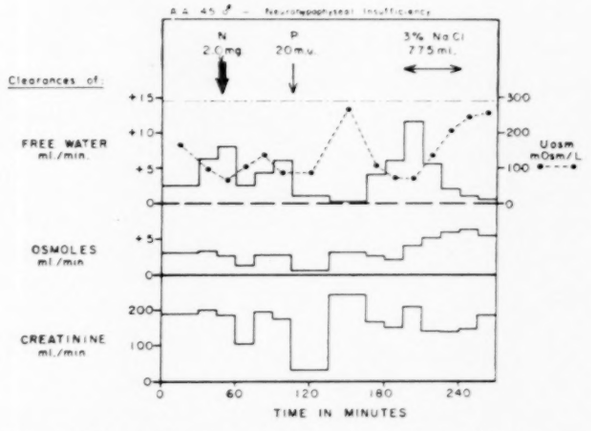

FIG. 3. Case III. Effect of nicotine and hypertonic saline on ADH secretion.

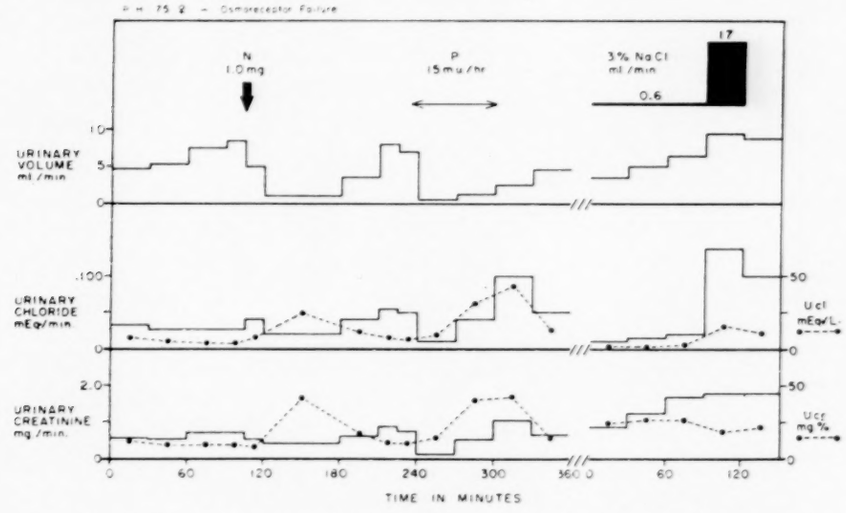

FIG. 4. Case IV. Effect of nicotine and hypertonic saline on ADH secretion.

0.47 indicated that slight antidiuresis had occurred. A moderate fall in creatinine excretion was observed during this period. C_{H_2O} increased and then declined during hypertonic saline solution infusion, and a marked increase in C_{osm} occurred. These observations suggested that some ADH was released with nicotine and with administration of hypertonic saline solution. The antidiuresis was less than that with 20 milliunits pitressin, since osmolar U/P rose to 0.97 with pitressin.

CASE IV. (Fig. 4.) This patient was a seventy-five-year old white woman with diabetes insipidus of seven years' duration treated with PTO, who had previously shown no antidiuresis with the hypertonic saline solution test. Extensive bilateral calcification of the internal carotid arteries was evident in skull roentgenograms. There were no symptoms or signs of anterior pituitary insufficiency and the basal metabolic rate was normal. Polyuria of 6 L. per day was evident when pitressin therapy was withdrawn. The antidiuresis which followed the administration of 1 mg. nicotine was equal to that with 15 milliunits pitressin administered intravenously over a one-hour period. The hypertonic saline solution test was performed on the following day. Because of an inability to achieve the level of diuresis observed the preceding day with initial hydration, intravenous injection of 3 per cent saline solution was instituted at the rate of 0.6 ml. per minute, to provide electrolyte for renal excretion [*16*]. A rise in urine flow occurred, and the saline solution was then administered rapidly at 17 ml. per minute for a thirty-minute period. Urine flow increased, U_{cr} decreased and U_{Cl} rose only slightly. These changes suggested that an increase in water excretion had occurred, and that ADH was not released with the hypertonic stimulus. Only a slight increase in chloride excretion occurred with administration of saline solution.

CASE V. (Fig. 5.) A twenty-six year old white woman had postpartum hemorrhage due to afibrinogenemia complicated by shock and lower nephron nephrosis. Anterior hypopituitarism (Sheehan's syndrome) and moderate polyuria subsequently developed.* Figure 5 depicts the results of a study conducted approximately one year after delivery, before hormonal therapy was begun, when the urine volume was approximately 2.5 L. per day, and the patient no longer had nocturnal thirst and nocturia. Following a nine-hour overnight period of dehydration, the urine concentrated to 457 mOsm/L., and C_{cr} was depressed to 49 ml. per minute. A prolonged antidiuresis lasting many hours followed the intravenous administration of 1 mg. nicotine, A slow infusion of 3 per cent saline solution was also given to this patient because of the persistent antidiuresis. U_{osm} thereafter declined and C_{H_2O} become positive. The rapid administration of hypertonic saline solution was associated with an increase in C_{H_2O} and a marked increase in C_{osm}. These observations demonstrated an excessive re-

* Detailed studies on the mechanisms of polyuria in this patient will be reported separately [*17*].

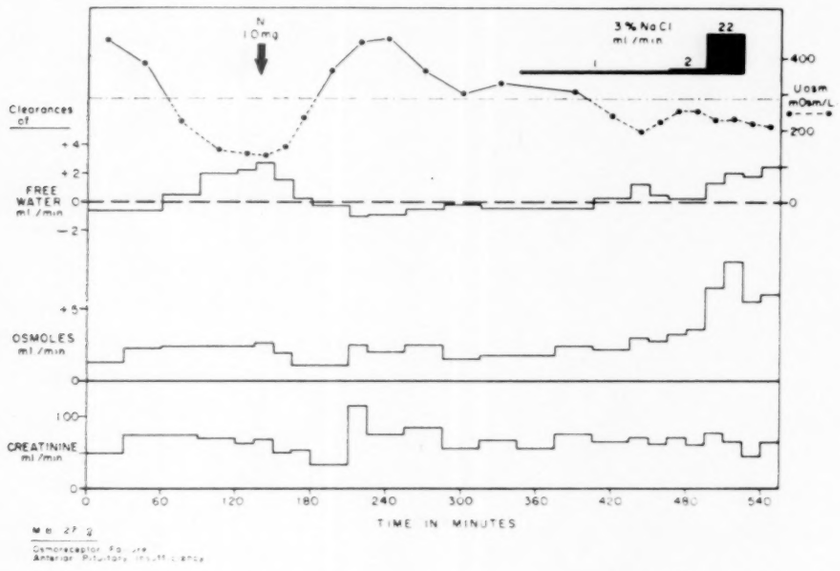

FIG. 5. Case V. Effect of nicotine and hypertonic saline on ADH secretion.

sponse to nicotine but an absent osmoreceptor response to hypertonicity. A normal renal response to pitressin had been demonstrated in previous studies.

CASE VI. A few months prior to the study, polydipsia and polyuria developed in a fifty

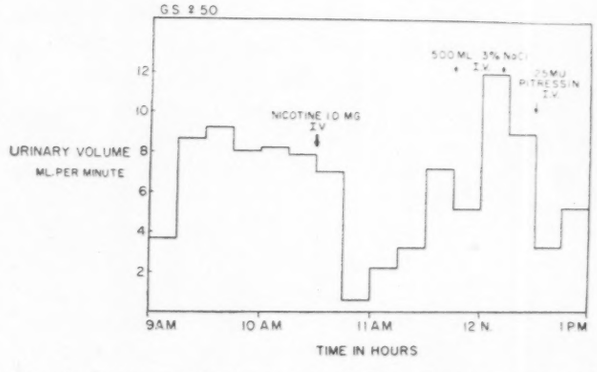

FIG. 6. Case VI. Osmoreceptor failure in carcinoma of the breast.

year old white woman with widespread metastatic carcinoma of the breast. The urine volume approximated 4 L. per day. Figure 6 depicts the changes in urine flow which were observed following administration of nicotine, hypertonic saline solution and pitressin.* A marked fall in urine flow occurred with the administration of 1 mg. nicotine. This was accompanied by profuse vomiting. Urine flow rose during and immediately following the 3 per cent saline solution infusion, and an antidiuresis followed the intravenous administration of 25 milliunits pitressin. A few weeks later the patient died of carcinoma. At necropsy the brain was found to be infiltrated with tumor in the region of the left temporal lobe, and the pituitary gland. Microscopic examination of the hypothalamic and pituitary regions showed extensive infiltration and loss of normal architecture of the entire posterior pituitary and pituitary stalk (Figs. 7 and 8) but the supraoptico-hypophyseal tract and the supraoptic and paraventricular nuclei were normal in appearance. (Fig. 9.) Sections of the hypothalamic and pituitary regions were fixed in Bouin's solution shortly after the patient's death. These were prepared with Gomori chrome alum-hemotoxylin stain [*18*] for demonstration of neurohypophyseal secretory material [*6,7*]. There was a complete absence of stainable secretory material in the posterior pituitary, stalk and infundibulum, which had been extensively destroyed by tumor. Examination of the Gomori-stained supraoptic and paraventricular nuclei revealed normal cellular architecture and an abundance of secretory granules within the cytoplasm of the ganglion cells. (Fig. 10.)

CASE VII. (Fig. 11.) A fifty-five year old man with chronic cholangiolitic hepatitis and malnutrition had polydipsia up to 10 L. per day

* The authors wish to acknowledge the assistance of Dr. Najib Abu-Hydar in the performance of this study.

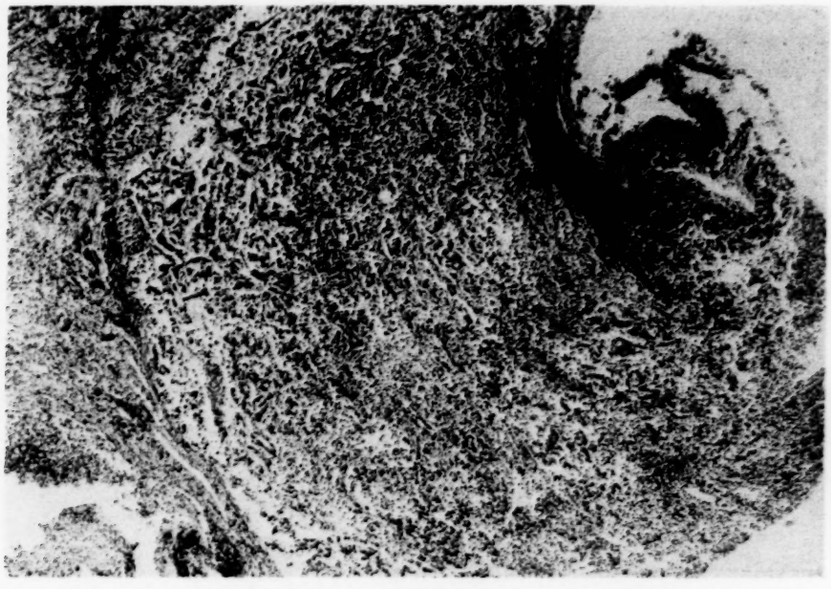

FIG. 7. Case VI. Showing Gomori-stained posterior pituitary gland completely destroyed by tumor cells and devoid of neurosecretory material.

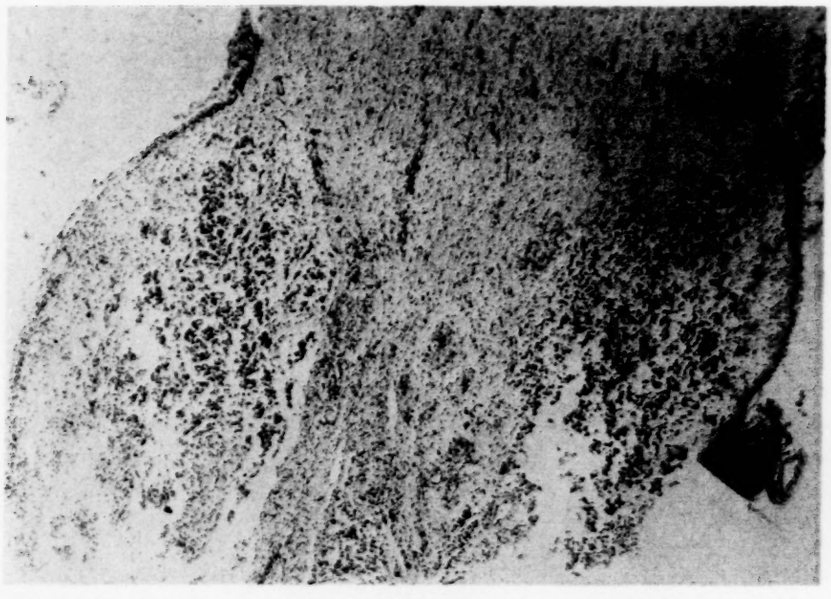

FIG. 8. Case VI. The pituitary stalk is extensively infiltrated by tumor cells.

approximately eight months prior to this study. Soon afterwards, dependent edema developed, which cleared spontaneously. The serum albumin was 2.5 gm. per 100 ml. and serum sodium was 131 mEq./L. at that time, and were similarly depressed at the time of study. The urine volume was 4 to 6 L. on the days immediately preceding study. Fluids were withheld from 10:00 P.M. the evening before study. The morning urine collected between 7:00 A.M. and 8:00 A.M. concentrated to a specific gravity of 1.013, with a total solute concentration of 434 mOsm/L. The patient was a regular cigarette smoker, and 3 mg. of nicotine was administered intravenously with no untoward symptoms save for a moderate "lightheaded" feeling; no antidiuresis was observed. A moderate antidiuresis occurred with the administration of 15 milliunits pitressin. There was a rise in C_{H_2O} when an infusion of saline solution was given, followed by a gradual

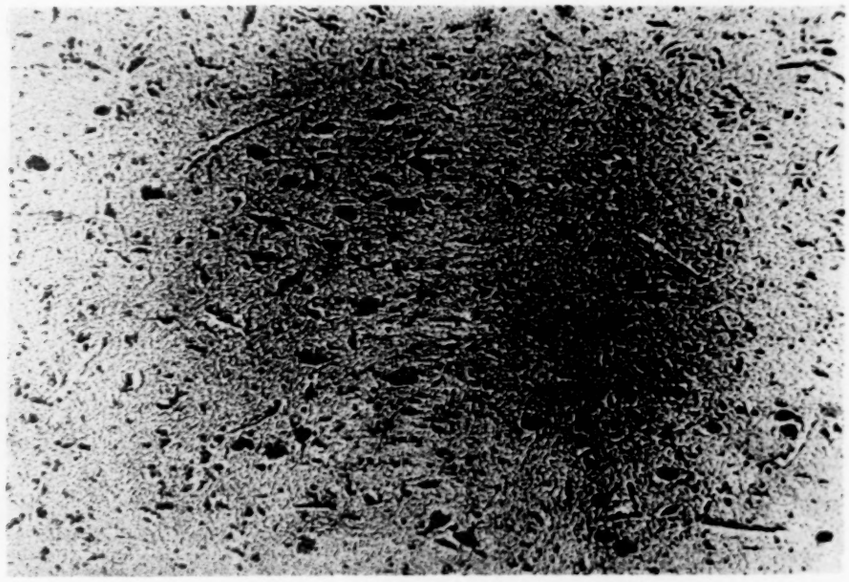

FIG. 9. Case VI. Gomori-stained section through supraoptic nucleus showing numerous normal appearing ganglion cells.

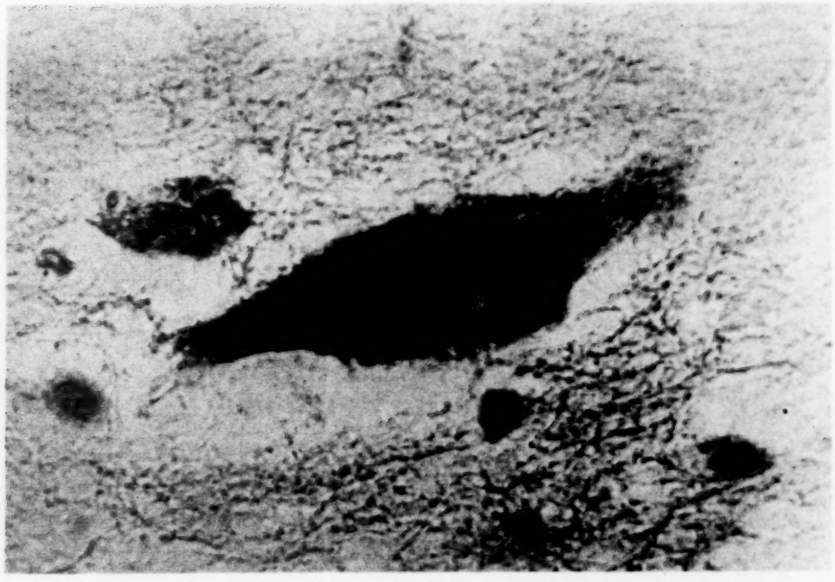

FIG. 10. Case VI. Showing a normal ganglion cell of the supraoptic nucleus filled with Gomori-stained neurosecretory material.

fall to a negative value one hour after the infusion had ended. An osmotic diuresis did not occur with the saline solution infusion. The findings were interpreted as representing a primary polydipsia with a questionably abnormal response to nicotine. The patient subsequently voluntarily restricted fluid intake without complaints of thirst, and polyuria disappeared.

CASE VIII. Infectious hepatitis developed in a thirty-seven year old white woman and she was treated with 200 mg. cortisone daily for a three-week period at another hospital. During the first week of therapy extreme thirst, blurring of vision and transient periods of blindness developed. Fluid intake rose to 6 to 7 L. daily. A craving for cigarettes and coffee soon followed and she smoked sixty cigarettes and drank twelve to

eighteen cups of coffee daily. A libidinous drive and extreme nervousness and hyperactivity became manifest, cortisone was discontinued, and she was transferred to the Peter Bent Brigham Hospital in a hypomanic state. The electroencephalographic pattern showed abnormal spikes in the frontal area. Cerebrospinal fluid examination and skull x-rays were normal. There were no visual field defects and the oculi fundi were normal on ophthalmoscopic examination. Liver function tests were within the normal range. Coffee and cigarettes were withheld after 10:00 P.M. the evening before study. A ten-hour period of dehydration yielded a maximum urinary specific gravity the next morning of 1.014. One mg. nicotine administered during a water diuresis produced minimal symptoms of dizziness and numbness. (Fig. 12.) The patient then smoked three cigarettes rapidly within fifteen minutes; this was followed by a second injection of 1.5 mg. nicotine intravenously. It was estimated that this patient received a total of 4.75 to 5.5 mg. nicotine within a thirty-minute period without evidence of toxicity or stimulation of the hypothalamic release of ADH, since no changes in the urine flow, U_{cr} or U_{Cl} were observed. There was a normal antidiuretic response to 15 milliunits pitressin and to hypertonic saline solution. A few weeks after study the hypomania and polydipsia subsided, and moderate depression ensued. Two months later a second study was performed. Within a fifteen-minute period 2.5 mg. nicotine were administered intravenously in divided doses and one cigarette was smoked; this produced slight nausea and dizziness. A 38 per cent decline in urine flow followed administration of nicotine but U_{cr} and U_{Cl} remained depressed, indicating that little or no ADH had been released. The response to 15 milliunits pitressin was again normal. A final study was performed four months later, at a time when the patient was more alert and active. There was some increase in water intake, with urine volumes of approximately 2 L. per day. The patient was given serial injections of nicotine at thirty-minute intervals of 1.0, 2.0, 3.0 and 5.0 mg. in an at-

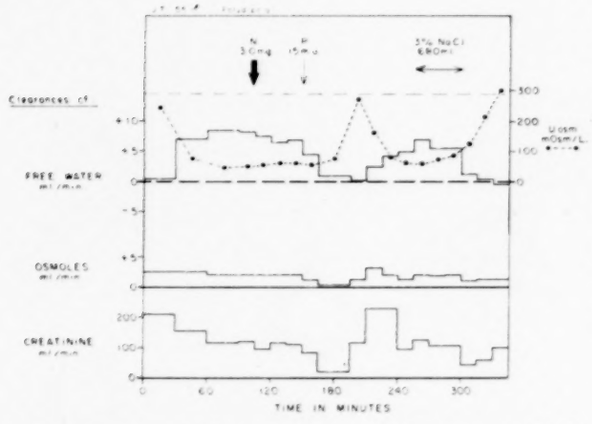

FIG. 11. Case VII. Effect of nicotine and hypertonic saline on ADH secretion.

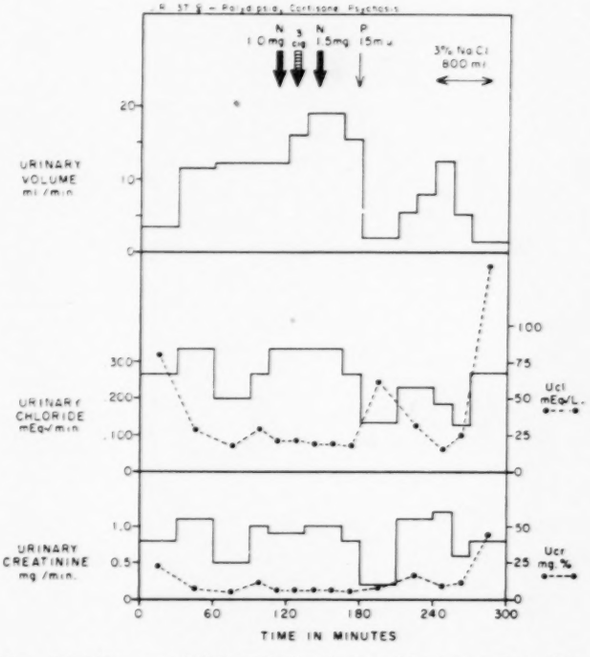

FIG. 12. Case VIII. Effect of nicotine and hypertonic saline on ADH secretion.

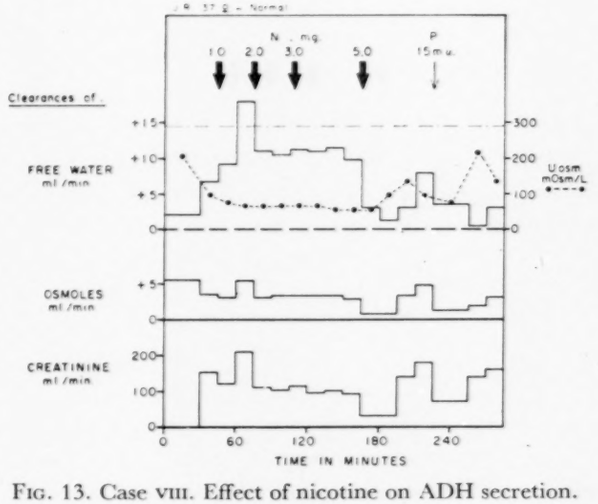

FIG. 13. Case VIII. Effect of nicotine on ADH secretion.

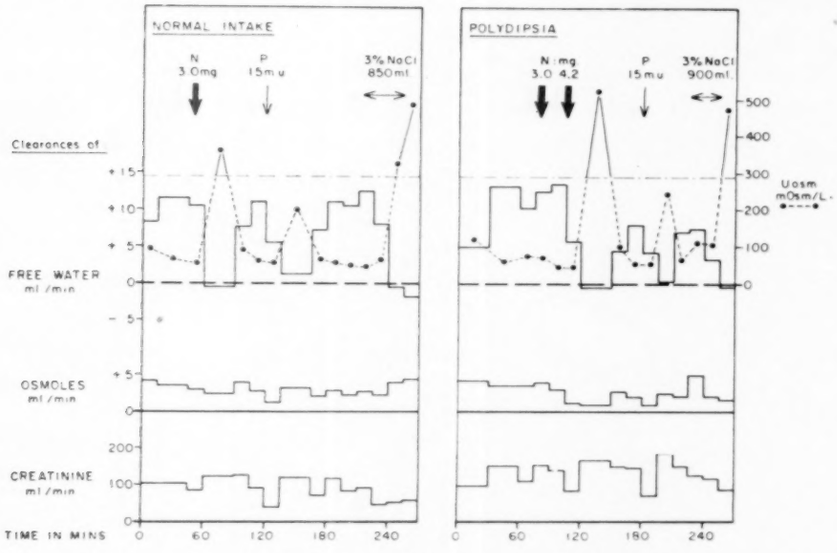

FIG. 14. Case IX. Effect of nicotine and hypertonic saline on ADH secretion in a patient with cyclical polydipsia and hypomania.

tempt to stimulate ADH secretion. (Fig. 13.) Moderate nausea was experienced with the 3.0 mg. dose, and profuse vomiting with the 5.0 mg. dose. Only with the larger dose was there a transient fall in C_{H_2O} but U_{osm} rose only slightly and a deep fall in C_{cr} occurred. A greater antidiuresis followed the administration of 15 milliunits pitressin. These changes suggested only minimal ADH release with massive hypothalamic stimulation.

CASE IX. (Fig. 9.) A thirty-seven year old white woman with manic-depressive psychosis experienced periodic attacks of anxiety and thirst. During states of panic, urine volumes ranged from 4 to 6 L. per day and urinary 17-hydroxycorticoid excretion [*19*] rose to high levels, declining again to normal values during quiet periods [*20*]. In a study performed during a relatively normal period, a normal antidiuretic response to 3 mg. nicotine was observed, accompanied only by slight dizziness. (Fig. 14.) The response to pitressin and saline solution were normal. Three months later infectious hepatitis developed in the patient. Her condition improved after six weeks of bed rest. Liver function tests thereafter were normal. Eight months after the first study, while in an excited polydipsic state, and when urinary steroid excretion was increasing, a second study was performed. (Fig. 14.) Three mg. nicotine produced no antidiuresis and slight dizziness was again noted. Thirty minutes later an infusion of 5.0 mg. nicotine was begun but severe nausea prompted discontinuance of infusion when 4.2 mg. had been given. A normal antidiuresis occurred with the increased dosage of nicotine. The responses to identical doses of pitressin and saline solution were unaltered.

COMMENTS

The antidiuretic action of nicotine has been well documented in human subjects [*11,12,21*] and animals [*11,22*]. This drug apparently stimulates the secretion of ADH from the neurohypophysis, and antidiuretic substances have been demonstrated in the urine following its administration [*23*]. There is little reason to doubt that the action of nicotine resembles the "nicotinic" action of acetylcholine which has been shown to stimulate ADH release following intravenous injection in the atropinized dog [*10*]. The threshold dose of nicotine in normal subjects is approximately 0.5 to 1.0 mg. for nonsmokers, and as much as 3 mg. for smokers [*21*], although many smokers respond to smaller doses [*11*]. In the present study nicotine was used as a hypothalamic stimulant to study the secretory activity of the neurohypophysis—the presence or absence of antidiuresis indicating the functional state of the hypothalamic nuclei responsible for ADH production. The frequent occurrence of nausea or vomiting served as distressing but reliable indicators of adequate central nervous system stimulation.

Two patients with diabetes insipidus showed

no antidiuresis with nicotine or saline solution administration, indicating a complete loss of neurohypophyseal function (Cases I and II). The absence of a nicotine response in the patient with Hand-Schüller-Christian disease suggested that complete retrograde degeneration of hypothalamic neurosecretory cells may have resulted from granulomatous invasion of the stalk [*24*] and prolonged interruption of the axones in the tract [*3*]. The etiology of neurohypophyseal failure in the second patient was obscure. The concomitant occurrence of diabetes mellitus was of interest, since the coexistence of both forms of diabetes is quite rare [*25*]. The third patient in this group showed minimal antidiuresis with nicotine and a moderate antidiuresis with hypertonic saline solution. These findings, coupled with the results of the dehydration test and the symptoms of polydipsia and polyuria, substantiated a clinical diagnosis of neurohypophyseal insufficiency.

The next three patients (Cases IV, V, VI) showed decided differences from the preceding subjects. All three had a significant antidiuresis following small doses of nicotine but gave no response to hypertonic saline solution. The normal nicotine antidiuresis indicated the presence of functioning neurosecretory tissue in the hypothalamus containing quantities of ADH sufficient to halt a water diuresis, which presumably should also be capable of maintaining normal water balance. Why, then, did these patients suffer from polyuria? Since the hypertonicity produced by 3 per cent saline solution is believed to stimulate an osmoreceptor which maintains an optimal state of bodily hydration by regulating ADH secretion [*18*], the absence of a saline antidiuresis suggested that these subjects suffered from a selective failure of osmoreceptor function, and were unable to secrete ADH despite serum hypertonicity. This condition ordinarily would be indistinguishable from primary neurohypophyseal failure if only the hypertonic saline solution test was performed.

The finding of an antidiuretic response to nicotine in diabetes insipidus had also been reported by Cates and Garrod [*21*] and Lewis and Chalmers [*12*]. None of the patients studied by the first group evidenced antidiuresis with small doses of nicotine, but a considerable number responded in some degree to larger doses of 5 to 7 mg., suggesting residual ADH stores which were released only after massive central nervous system stimulation. Many of the patients reported by Lewis and Chalmers, however, had antidiuresis of varying degrees with small doses of nicotine. These authors attempted to quantify the antidiuretic response, and concluded that the majority of their patients had significant neurohypophyseal insufficiency, since the nicotine antidiuresis was equivalent to less than 10 milliunits pitressin in most subjects. One of their patients, however, had an antidiuresis to nicotine equivalent to 100 milliunits pitressin but no antidiuresis with hypertonic saline solution or with fluid deprivation. The results of their study resemble those obtained in the three patients herein reported, except that quantitation of the amount of ADH released with nicotine was not attempted in the present report. The fact that the patients reponded to small intravenous doses of pitressin with an antidiuresis which was approximately equal in magnitude to that observed with nicotine seemed sufficient evidence that this amount of ADH should be capable of maintaining satisfactory fluid balance in these patients. Polyuria due to ADH deficiency, therefore, should not have existed if control of the secretion of available ADH presumably present in the hypothalamus was normal.

All three subjects had other complications which may have been responsible for the failure of ADH secretion with osmoreceptor stimulation. One patient showed extensive bilateral calcification of the internal carotid arteries, and may also have had a vascular abnormality involving the osmoreceptor. The second subject suffered an infarction of the adenohypophysis secondary to postpartum hemorrhage and shock (Sheehan's syndrome), which occasionally may involve the posterior pituitary as well [*26*]. Polyuria was evident soon after delivery, at a time when a normal nicotine and pitressin antidiuresis but an absent response to hypertonic saline solution were observed. Polyuria abated somewhat when symptoms of anterior pituitary insufficiency supervened. Study at this time revealed increased sensitivity to the antidiuretic effect of nicotine, yet the defect in osmoreceptor response persisted. This observation suggested that the decrease in polyuria was not due to restoration of osmoreceptor function.

The last subject, who died of metastatic carcinoma, was found to have extensive infiltration and destruction of the posterior pituitary and stalk by tumor cells. The hypothalamus was intact. Interpretation of these findings according

to the neurosecretory theory of Scharrer [*4,5*] and Bargmann [*6,7*] suggests that adequate stores of ADH were present in these hypothalamic centers and were released with nicotine stimulation presumably into the hypothalamic circulation. The polyuria and the absent response to hypertonic saline solution were evidence that secretion of this ADH was not under osmoreceptor control. This patient received very little pitressin therapy and polyuria was constantly present prior to death, fluid therapy being maintained by parenteral administration when oral intake was unsatisfactory. The absence of neurosecretory granules from the posterior pituitary region, therefore, probably was not due to rapid secretion of ADH into the blood stream, since an accumulation of these granules has been observed during overhydration and depletion of stored material occurs only with prolonged dehydration [*27*]. These observations suggested that the osmoreceptor either may be located in the posterior pituitary or stalk and was also destroyed by tumor, or may require an intact posterior pituitary gland for transmission of secretory impulses. An alternate hypothesis is that no such structure exists and that hypertonic saline solution entering the hypophyseal circulation may merely promote the secretion into the blood stream of ADH which has accumulated about blood vessels in the posterior pituitary region. Destruction of the posterior pituitary gland or involvement of its blood supply may prevent ADH accumulated cephalad to this region from being secreted by its usual route, and a diabetes insipidus-like syndrome may result.

Other hypotheses may explain these findings. It is possible that intravenously administered 3 per cent saline solution is not as powerful a stimulus to the neurohypophysis as is nicotine. Also, chronic polydipsia may alter the response to hypertonic saline solution.

In the present study, the antidiuresis to saline solution was approximately equal in magnitude to the nicotine antidiuresis, suggesting that both stimulants, when given in adequate dosage, have similar degrees of activity on the neurohypophysis. Chronic water excess alone does not abolish the antidiuretic response to hypertonic saline solution, since normal results were observed in our patients with psychogenic polydipsia, as well as by Carter and Robbins [*28*]. A hypertonic expansion of plasma volume with the rapid infusion of hypertonic saline solution [*29*] theoretically may alter the osmoreceptor response. This factor probably did not play a role since other subjects given hypertonic saline solution demonstrated normal antidiuretic responses, and rapidly administered hyperoncotic albumin does not produce an antidiuresis in patients with diabetes insipidus, but does so in normal subjects [*30*]. The apparent failure of hypothalamic ADH to enter the systemic circulation and prevent development of diabetes insipidus has not been explained. The antidiuresis with overnight dehydration in the patient with Sheehan's syndrome and absent osmoreceptor function would suggest that fluid deprivation and contraction of body water content may represent a powerful stimulus for ADH secretion directly from the hypothalamus by mechanisms not entirely dependent on osmotic stimulation. The antidiuresis with dehydration could also be ascribed to a renal concentrating ability, apparently not dependent on ADH, which appears to be brought into play by a critical depression of glomerular filtration rate [*31*]. It is probable, therefore, that a selective failure of osmoreceptor function may be responsible for a diabetes insipidus-like syndrome in certain patients in whom hypothalamic neurosecretory tissue producing adequate amounts of ADH is apparently preserved. The factors predisposing to this failure of secretion of ADH have not been clarified, but may depend on posterior pituitary damage or vascular insufficiency resulting in destruction of the site for storage of preformed hormone or interruption of the normal pathway for secretion of ADH.

The relative resistance of the patients with polydipsia to nicotine stimulation warrants additional comment. In the first subject (Case VIII) the lack of toxic symptoms suggested that the hypothalamic threshold had not been reached, although most normal subjects respond to this dose, frequently without marked toxicity. In the patient with polydipsia associated with cortisone therapy, persistent unresponsiveness to large doses of nicotine suggested hypothalamic damage. Hypothalamic lesions, particularly in the region of the supraoptic nuclei, have been observed in experimental animals given large doses of cortisone [*32*]. The normal response to hypertonic saline solution indicated that ADH production by the hypothalamic neurones and the responsiveness of the neurohypophysis to osmotic stimulation were not involved. A functional disturbance of adjacent thirst-regulating centers in the hypothalamus

produced by massive doses of cortisone may have been responsible for polydipsia despite adequacy of the neurohypophyseal antidiuretic system. An obscure relationship between an abnormality in the nervous system control of the neurohypophysis and an aberration in thirst perception is suggested by these findings. The amelioration of the polydipsia with dissipation of the hypomania would relate polydipsia and the psychologic disturbance to the toxic effects of cortisone on the central nervous system. The third patient gave added evidence of a relationship between central nervous system excitability, polydipsia and an increased threshold of the hypothalamus to nicotine stimulation. The associated elevation of the adrenocortical secretion suggested a possible reciprocal relationship between the adrenal cortex and the hypothalamic control of ADH secretion which has been further explored [*33*]. These observations suggest that adrenal steroids may somehow be responsible for development of polydipsia in subjects with an intact neurohypophysis. The abnormal response to nicotine may be a manifestation of corticoid effect on hypothalamic thirst centers and the central nervous system control of ADH secretion, but may also reflect a vegetative disturbance in patients with primary polydipsia independent of the adrenal cortex and the neurohypophysis. The intimate anatomic relationship between thirst centers and the supraoptic nuclei [*34*] would suggest that adrenal steroids may exert important influences on the activity of this area of the hypothalamus. Since one patient with polydipsia had active liver disease, and the other two had recently recovered from hepatitis, further investigation of the role of the liver in these abnormal findings appears to be warranted.

SUMMARY

The functional state of the neurohypophyseal-renal system in patients with polyuria has been evaluated by serial intravenous administration of nicotine, hypertonic saline solution and pitressin under constant water-loading conditions. Changes in free water clearance following stimulation of the hypothalamic nuclei, osmoreceptors and renal tubules, respectively, have been measured as a convenient and reliable clinical index of ADH activity. Patients lacking antidiuretic responses to the administration of nicotine and hypertonic saline solution were presumed to have neurohypophyseal failure. Three subjects with posterior pituitary damage or vascular disease responded normally to small doses of nicotine but had no antidiuresis with saline solution administration. A selective failure of osmoreceptor function may be postulated to account for polyuria since hypothalamic neurosecretory tissue producing ADH was probably still preserved. The hypothesis was made from these observations that the "osmoreceptor" may reside in the posterior pituitary or stalk, rather than in the supraoptic neurones [*35*], or that no such structure exists and ADH stored in the perivascular regions of the posterior pituitary may be released directly into the blood stream when blood perfusing the gland becomes hypertonic. Destruction of the posterior lobe, by removing the normal route for storage and delivery of ADH, may result in a diabetes insipidus-like syndrome despite preservation of hypothalamic neurosecretory function.

Three patients with psychogenic polydipsia demonstrated abnormal responses to nicotine and normal responses to saline solution. The occurrence of polydipsia and hypomania during cortisone therapy in one patient, and polydipsia, agitation, elevation of threshold of the hypothalamus to nicotine, and increased adrenal cortical secretion in a second patient suggested a relationship between central nervous system excitability and/or adrenal steroid excess and disturbances of hypothalamic function. The antidiuretic response to nicotine appears to be a useful means of assessing hypothalamic function in man.

Acknowledgments: The authors wish to express their gratitude to Dr. Kendall Emerson, Jr. for permission to study his patients, and to Drs. Sanford Gifford and Edward Koudaraskas for psychiatric interpretations.

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Malignant Lymphoma and Lymphatic Leukemia Associated with Myeloma-Type Serum Proteins*

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Serum protein abnormalities, similar to those which are commonly found in multiple myeloma, have been observed occasionally in patients with malignant lymphoma and lymphatic leukemia [*1–7*]. One of us (E. F. O.) has previously reported two such cases in detail [*8*] and has presented a preliminary report [*9*] on five cases which are included in this paper.

The purposes of the present study are: (1) to report thirteen cases of malignant lymphoma and lymphatic leukemia, including one case of Hodgkin's disease, associated with myeloma-type serum proteins; and (2) to establish histopathologic criteria which would be of help in differentiating this group of cases from the usual variety of malignant lymphoma. The term "malignant lymphoma" is used here in a broad sense to embrace a group of disorders designated as reticulum cell sarcoma, lymphosarcoma and Hodgkin's disease.

MATERIAL AND METHOD

The subjects of this study were thirteen patients, admitted to the Francis Delafield Hospital and the Presbyterian Hospital between September, 1950 and June, 1956, in whom the diagnosis of malignant lymphoma and lymphatic leukemia was confirmed by tissue biopsies and/or bone marrow aspiration and peripheral blood studies. Each of the thirteen patients showed myeloma-type serum protein components on routine filter paper electrophoresis. Seven of the thirteen patients died. Autopsies were performed on four patients and are reported in detail along with two other cases of special interest. In nine cases tissue sections were available for study from one or several lymph nodes, bone marrow and other tumor sites. In four cases only bone marrow aspirations were available for study. All cases are summarized in Table 1.

An effort was made to search for histologic features differentiating this group of thirteen cases from the usual variety of malignant lymphoma and lymphatic leukemia. For this reason a control group of twenty-five patients with malignant lymphoma and leukemia on whom autopsies were performed consecutively was studied. During life all the patients in this control group showed normal or non-specifically abnormal serum electrophoretic patterns. Sections from multiple tumor sites, lymph nodes and bone marrow were available from all twenty-five autopsy subjects.

The tissues were fixed in Zenker's solution or 10 per cent formalin and stained with hematoxylin and eosin. Wilder silver reticulin impregnation, Hotchkiss periodic acid-Schiff technic for polysaccharides, and Unna-Pappenheim (methyl green-pyronin Y)‡ stain for plasma cells were also used.

The degree of plasmacytosis was evaluated in all available sections. An estimate of the proportion of plasma cells to the total number of lymphoid cells was recorded as follows: 0, plasma cells absent or rare; ±, 1 to 5 per cent; +, 5 to 10 per cent; ++, 10 to 25 per cent; +++, 25 to 50 per cent.

Serum electrophoresis was performed by the modified filter paper system described by Osserman and Lawlor [*8*], and the patterns of five representative cases of the study group of thirteen are reproduced in Figure 1.

CASE REPORTS

Case 1. J. C. (P.H. 751226), a seventy year old white man, was admitted with frequency, dysuria and hematuria of several months' duration. On examination, there was a large hard mass occupying the right loin but no lymphadenopathy or hepatosplenomegaly was noted.

‡ We have used Pyronin Y, National Aniline Division, Cert. No. NP 15. Samples of pyronin B and the dye supplied by the National Aniline Division, labelled Pyronin Y, Cert. No. NP 11, have been found unsatisfactory.

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TABLE I

CLINICAL AND ELECTROPHORETIC FINDINGS IN THIRTEEN PATIENTS WITH MALIGNANT LYMPHOMA AND LEUKEMIA EXHIBITING MYELOMA-TYPE SERUM ABNORMALITIES

Case No., Patient, Age and Sex	Clinical Data	Total Serum Protein (gm. %)	Electrophoretic Mobility of Abnormal Component
I. J. C. (751226), 70, M	P.H. admission 2/16/56 with dysuria, several months. Mass in right loin. No lymphadenopathy. Hemoglobulin 13.8 gm. %. White blood cells 8,000 per cu. mm. Active bone marrow. No myeloma cells. 2/27/56, biopsy bladder, reticulum cell sarcoma. Treated with x-ray and prednisone. Died at home, 5/19/56, following myocardial infarction. Autopsy performed.	6.2	Slow gamma
II. D. R. (148183), 66, M	P.H. admission 7/28/53 with perirectal pain and dysuria of one year. Myelogram: block at L_3. Hemoglobin 16.7 gm. %. White blood cells 10,950 per cu. mm. 8/14/53, laminectomy and partial resection of epidural tumor. Diagnosis: lymphosarcoma, lymphocytic type. Condition stationary on 3/9/56, following course of x-ray.	6.9	Slow gamma
III. J. R. (007609), 59, M	P.H. admission 9/8/50 with weakness, loss of weight two years. In 1948, hemorrhage from tonsils after instrumentation; 1949, hemorrhage and fistula-in-ano following hemorrhoidectomy. Enlarged matted peripheral lymph nodes. Hepatosplenomegaly. Hemoglobin 7 gm. %. White blood cells 5,950 per cu. mm. Marrow, 38% lymphocytes. Biopsy axillary nodes: lymphosarcoma, lymphocytic type. Treated with blood, x-ray to spleen, cortisone, TEM. Condition fair 5/7/56.	8.0	Fast gamma
IV. D. G. (6988), 51, M	F.D.H. admission 12/12/55 with lump in right supraclavicular region of one year. Hemoglobin 12.8 gm. %. White blood cells 8,450 per cu. mm. Marrow active, erythroid hyperplasia; eosinophilic myelocytes 7%, lymphocytes 16%, plasma cells 1%. Biopsy supraclavicular node: lymphosarcoma, lymphocytic type. Retrosternal mass demonstrated by x-ray. Good response to x-ray therapy to right supraclavicular region and superior mediastinum, 5/17/56.	6.9	Slow gamma (12/6/55)
V. T. C. (3334), 59, F	F.D.H. admission 2/15/54 with painful submandibular swelling of three months, followed by generalized lymphadenopathy. Hepatosplenomegaly. Hemoglobin 6.1 gm. %. White blood cells 5,500 per cu. mm. Biopsy supraclavicular node: lymphosarcoma, lymphocytic type. Treated with transfusions, TEM, CB 1,348. Died 12/23/54. Autopsy performed.	6.8	Intermediate between gamma and beta
VI. B. M. (3447), 50, M	F.D.H. admission 4/28/54 with weakness, low back pain, right lateral chest pain. No lymphadenopathy or hepatosplenomegaly. Skeletal survey: disseminated osteolytic mottling; compression of L_1. Hemoglobulin 6.0 gm. %. White blood cells 6,900 per cu. mm. Marrow aspirations: replacement by young lymphoblasts, no plasma cells. Diagnosis: lymphosarcoma, lymphocytic type. Treated with NH_2 and x-ray to spine. Died at home on 11/7/54.	7.7	Fast gamma
VII. M. K. (6365), 56, F	F.D.H. admission 5/12/56 with low back pain for three years. Anemia (8 gm. %), albuminuria with positive Bence Jones protein in urine discovered in 1952. Poorly defined right upper quadrant mass. Hemoglobin 4.8 gm. %. White blood cells 5,800 per cu. mm. Non-protein nitrogen 100 mg. %. Skeletal survey: demineralization and varying degrees of collapse of vertebral bodies. Bone marrow: heavy infiltration by lymphocytes (80%), consistent with lymphosarcoma. Treated with transfusions, prednisone, CB 1348. 6/4/56: Hemoglobin 7.6 gm. %, condition improved.	17.0	Fast gamma

TABLE 1 (*Continued*)

CLINICAL AND ELECTROPHORETIC FINDINGS IN THIRTEEN PATIENTS WITH MALIGNANT LYMPHOMA AND LEUKEMIA EXHIBITING MYELOMA-TYPE SERUM ABNORMALITIES

Case No., Patient, Age and Sex	Clinical Data	Total Serum Protein (gm. %)	Electrophoretic Mobility of Abnormal Component
VIII. V. H. (5652), 65, M	F.D.H. admission 4/3/56 with pain in region of right medial malleolus of one year. Generalized lymphadenopathy. Hepatosplenomegaly. Hemoglobin 9.2 gm. %. White blood cells 7,650 per cu. mm. (50% lymphocytes). Marrow cellular, 63% lymphocytes, no plasma cells. Biopsy of several nodes: (?) lymphosarcoma. Treated with x-ray to right ankle, CB 1348. 6/4/56: condition stationary, comfortable.	10.8	Two components: slow and fast gamma
IX. J. T. (4977), 57, M	F.D.H. admission 7/13/55. Splenomegaly discovered in 1952 during routine checkup. Hemoglobin 10.2 gm. %. White blood cells 7,350 per cu. mm. (73% lymphocytes). Marrow aspirations: 42 to 55% lymphocytes, 0–1% plasma cells. Diagnosis: chronic lymphocytic leukemia, subleukemic stage. Transient improvement after x-ray, cortisone, CB 1348. Died 10/9/55. Autopsy not granted.	5.0	Slow gamma
X. S. K. (4421), 62, F	F.D.H. admission 6/27/54 with epistaxis. Enlarged cervical nodes since 1953. Hemoglobin 4.8 gm. %. White blood cells 16,500 per cu. mm. (73% lymphocytes). Biopsy cervical node consistent with chronic lymphocytic leukemia. Treated with cortisone. Died 12/19/55 after falling out of bed. Autopsy performed.	7.7	Slow gamma
XI. A. G. (4655), 60, M	F.D.H. admission 11/9/54 with weakness one year. Cervical lymphadenopathy. Hepatosplenomegaly. Hemoglobin 6.2 gm. %. White blood cells 12,000 per cu mm. (68% lymphocytes). Biopsy of cervical node: Hodgkin's disease. Treated with Thio-tepa. Died 12/9/54. Autopsy performed.	5.4	Fast gamma
XII. C. L. (202085), 67, M	P.H. admission 2/6/52 with cervical lymphadenopathy. Hemoglobin 6.5 gm. %. White blood cells 224,000 per cu. mm. (99% lymphocytes). Bone marrow aspiration compatible with chronic lymphocytic leukemia. Died 12/29/54 presumably from myocardial infarction. No autopsy obtained.	7.8	Mid gamma
XIII. J. S. (490086), 75, M	Seen in P.H. Clinic in January 1955 with 1 to 2 years of fatigue and occasional epistaxes. Hepatosplenomegaly, no lymphadenopathy. Hemoglobin 10 gm. %. White blood cells 21,000 per cu. mm. (54% lymphocytes). Bone marrow aspirations compatible with chronic lymphocytic leukemia. Treated with transfusions, prednisone and x-ray. On 4/3/56 severe jaundice reported. Condition stationary 6/5/56.	7.6	Intermediate between gamma and beta

NOTE: P.H. = Presbyterian Hospital. F.D.H. = Francis Delafield Hospital.

Laboratory data revealed the following: Hemoglobin, 13.8 gm. per cent; white blood cells, 8,000 per cu. mm.; normal differential count; serum protein, 6.2 gm. per cent with abnormal gamma component on electrophoresis. (Fig. 1.) Bone marrow was active and contained no "myeloma cells." An intravenous pyelogram demonstrated absence of dye excretion on the right. The bladder was moderately thickened and pushed upward. A presacral air study revealed invasion of the right psoas muscle by tumor. In February, 1956, cystoscopy and transurethral biopsy of a thickened bladder wall were performed. A diagnosis of reticulum cell sarcoma was made. The patient improved slightly following x-ray therapy to the bladder and a course of prednisone. He died at home following a myocardial infarction.

At autopsy, there was thrombosis of the right coronary artery and myocardial infarction. Tumor

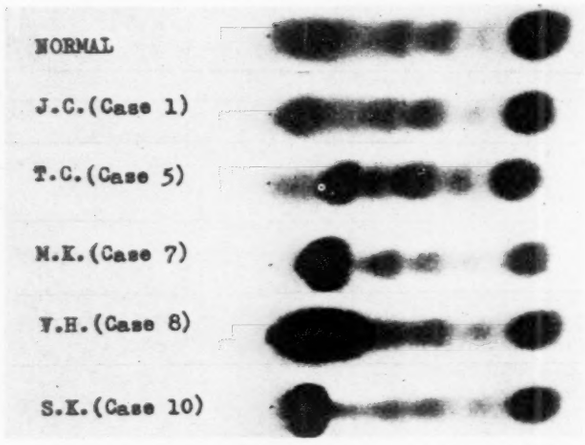

FIG. 1. Paper electrophoretic patterns of the serum proteins of five patients with malignant lymphoma and chronic lymphatic leukemia showing abnormal gamma and intermediate components which are usually observed in multiple myeloma.

infiltrated the wall of the bladder and extended retroperitoneally. It encircled both ureters and invaded the right kidney which was hydronephrotic. The skeleton was free of disease. Histologic examination of tissues from the bladder, retroperitoneum and right kidney demonstrated invasion by anaplastic cells. (Fig. 2.) There was no tendency to form sheets or glands. The anaplastic cells exceeded twice the size of mature lymphocytes and resembled reticulum cells. The nuclei were relatively large, dense, pleomorphic and contained one or several prominent nucleoli. Mitoses averaged 1 per high power field. The cytoplasm was moderately abundant and basophilic. It showed a strong affinity for pyronin. The Wilder stain demonstrated in some areas a reticulum pattern which was characteristic of reticulum cell sarcoma. No mature plasma cells were present. Sections of the vertebrae showed a normal osseous pattern and normal hematopoietic activity; no tumor cells or plasma cells were recognized.

This case illustrates extraskeletal involvement by a tumor with the histologic characteristics of reticulum cell sarcoma. The marked pyroninophilia of the cytoplasm of the tumor cells is noteworthy. The apparent similarity between anaplastic reticulum cells and myeloma cells has impressed Bayrd [*10*] and Snapper [*11*]. Snapper, Turner and Moscovitz [*11*] cite two patients reported to have reticulum cell sarcoma by biopsy of lymph nodes. At autopsy, they were found to have an infiltration by "wildly growing, invasive cells, which resembled reticulum cells in some areas and mature myeloma cells in other areas." Bayrd [*10*] has reported the case of a sixty year old woman with osteolytic lesions, Bence Jones proteinuria and hepatosplenomegaly in whom a leukemic invasion of the peripheral blood with cells indistinguishable from reticulum cells terminally developed.

CASE V. T. C. (F.D.H. 3334), a fifty-nine year old white woman, was admitted with a painful right submandibular swelling which had first been noted in December, 1953. Examination revealed generalized lymphadenopathy and hepatosplenomegaly.

Laboratory data revealed the following: Hemoglobin, 6.1 gm. per cent; white blood cells, 5,000 per cu. mm., normal differential count; serum protein, 6.8 gm. per cent. There was marked increase in an electrophoretically homogeneous abnormal gamma component. (Fig. 1.) X-rays of the skeleton revealed no abnormalities. A biopsy specimen of a supraclavicular lymph node showed a lymphosarcoma of the lymphocytic type. Many plasma cells were scattered among lymphocytes, which formed the predominant cell type. (Fig. 3.) Chlorambucil (CB 1348) and TEM therapy were without benefit and the patient died in December, 1954.

At autopsy, there was a generalized enlargement of the lymph nodes. The spleen was normal in size. The liver weighed 1,400 gm. The bone marrow was abundant and was deep brown in color. Most tissues examined showed lymphoid infiltration accompanied with fibrosis. Plasma cells were intermingled among lymphocytes in the bone marrow and spleen. The lamina propria of the stomach was heavily infiltrated by plasma cells. (Fig. 4.) Plasma cells were less conspicuous in the lymph nodes at autopsy. A review of the biopsy sections showed a marked increase in the number of plasma cells which in some areas constituted up to 25 per cent of the total number of cells.

CASE VII. M. K. (F.D.H. 6365), a fifty-six year old white woman, was found to be severely anemic and noted to have Bence Jones proteinuria in 1952. Bone pains developed in 1952. A diagnosis of multiple myeloma was made in another hospital in 1955 following a bone marrow aspiration. On admission to this hospital in May, 1956, the peripheral lymph nodes, liver and spleen were not enlarged. There was tenderness over the ribs and spine.

Laboratory data revealed the following: Hemoglobin, 4.8 gm. per cent; white blood cells 5,800 per cu. mm., normal differential; serum protein, 17 gm. per cent. Serum electrophoresis showed a massive amount of an abnormal homogeneous protein with fast gamma mobility. (Fig. 1.) The proteinuria consisted of albumin, a peak with the mobility of the abnormal serum component and another homogeneous component with a beta mobility. X-rays of the spine demonstrated extensive demineralization and varying degrees of collapse of almost all vertebral bodies. Bone marrow aspirations revealed marked cellularity and a heavy infiltration by lymphoid cells (80 per cent). Many lymphoid cells were large and contained a relatively abundant cytoplasm which showed a strong affinity for pyronin. (Fig. 5.) No typical myeloma cells and no increase in plasma cells were noted.

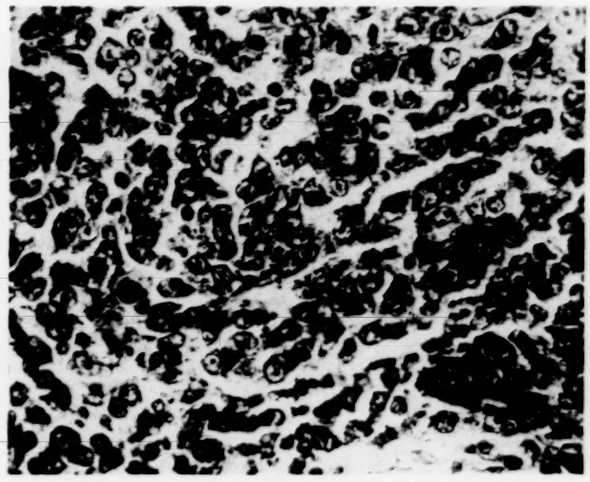

FIG. 2. Case I, J. C. Anaplastic cells in wall of urinary bladder. The Wilder stain showed a reticulum pattern characteristic of reticulum cell sarcoma. The cytoplasm showed a strong affinity for pyronin, as in plasma cells and myeloma cells. (Hematoxylin and eosin; original magnification, × 430.)

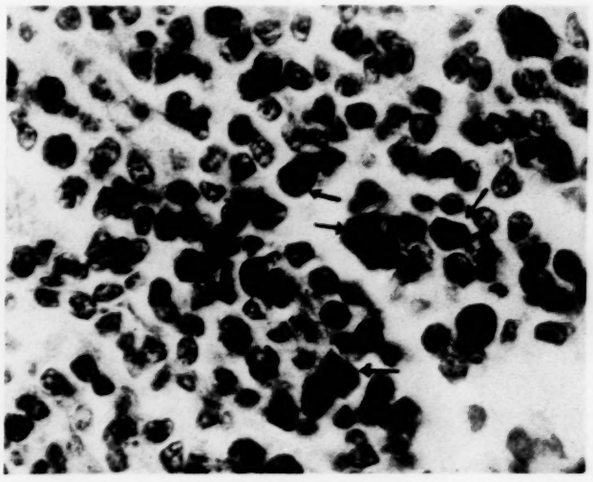

FIG. 3. Case V, T. C. Supraclavicular lymph node. Plasma cells (arrows) are scattered among lymphocytes which form the predominant cell type. (Methyl green-pyronin Y; original magnification, × 900.)

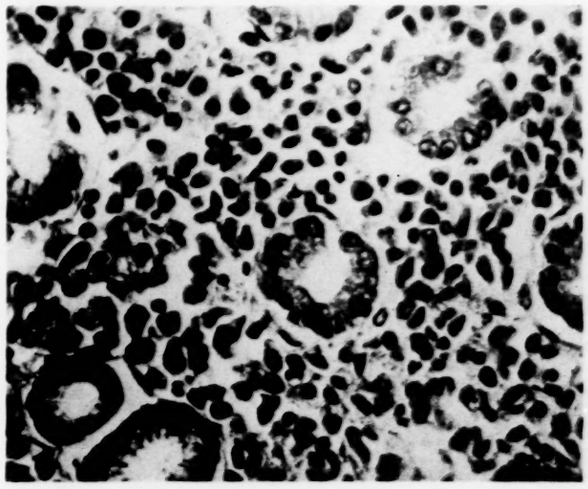

FIG. 4. Case V, T. C. (Cf. Fig. 3.) Plasma cells are present in great number in the lamina propria of the stomach. This is the only instance in this study where the majority of the infiltrating cells in one region of the body were plasma cells. (Hematoxylin and eosin; original magnification, × 430.)

This situation is duplicated in another patient (No. VI), who also exhibited the clinical and radiologic picture of multiple myeloma. Repeated bone marrow aspirations disclosed a predominance of lymphoid cells (90 per cent). No mature plasma cells were present but occasionally large lymphoid cells resembled myeloma cells. (Fig. 6.)

CASE VIII. V. H. (F.D.H. 5652), a sixty-five year old white man, had generalized lymphadenopathy in 1952. Repeated biopsy specimens of peripheral nodes were reported as (?) lymphosarcoma or reactive hyperplasia of lymph nodes. With neither local radiotherapy nor systemic tumor chemotherapy, the patient remained asymptomatic until April, 1956 when he was admitted to the hospital with pain in the region of the right medial malleolus. Physical examination revealed generalized lymphadenopathy, hepatosplenomegaly, tenderness and induration in the region of the right medial malleolus.

Laboratory data revealed the following: Hemoglobin, 9.2 gm. per cent; white blood cells 7,650 per cu. mm., with 50 per cent lymphocytes. Serum proteins varied between 10.8 and 11.2 gm. per cent. On electrophoresis there was marked increase in a slow and another fast gamma component. (Fig. 1.) A bone marrow aspiration yielded 63 per cent lymphocytes and no plasma cells.

Biopsies of the lymph nodes were performed between 1952 and 1956. All demonstrated partial to complete obliteration of the follicular pattern with a diffuse and dense infiltration by apparently mature lymphocytes. These also infiltrated the capsule and pericapsular stroma. In some sections there was partial retention of lymphoid follicles which displayed a marked reticulum cell hyperplasia in their center. Plasma cells constituted roughly 10 per cent of the total number of cells, and transitional forms between lymphocytes and plasma cells were frequently encountered. (Fig. 7.) Biopsy specimens of the skin, subcutaneous tissue and periosteum of the region of the right medial malleolus showed infiltration by lymphoid cells mixed with an almost equal number of relatively mature plasma cells. There was, in addition, hyalinization of parts of the stroma. These hyalinized areas and the subendothelial areas of some vessels took the Congo red stain but did not show any metachromasia with the methyl violet stain. The overall picture was that of a lymphosarcoma associated with marked plasmacytosis and paramyloidosis.

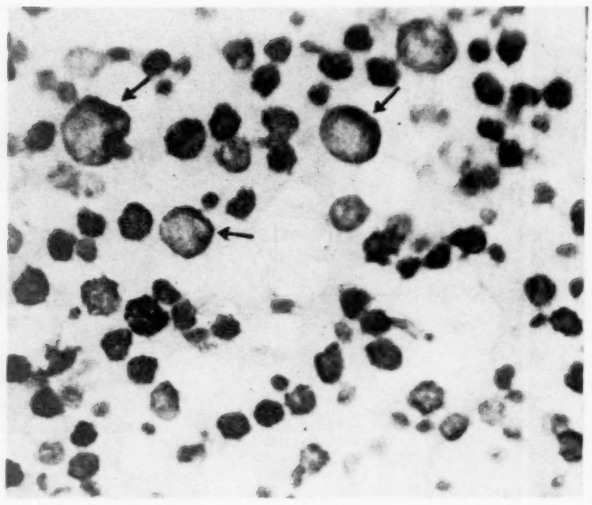

FIG. 5. Case VII, M. K. Large lymphoid cells (arrows) in the bone marrow showing a strongly pyroninophilic cytoplasm. (Methyl green-pyronin Y; original magnification, × 900.)

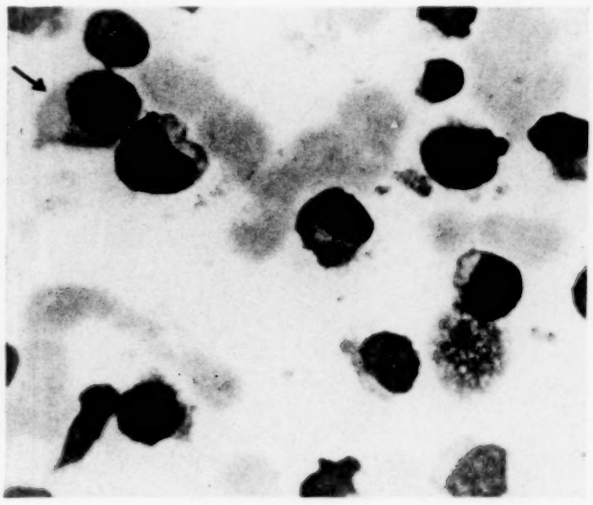

FIG. 6. Case VI, B. M. A cell in the left upper quadrant has the characteristics of a myeloma cell. Young lymphoblasts and large lymphoid cells with an abundant basophilic cytoplasm constitute over 90 per cent of the cellular elements in the marrow. No mature plasma cells are present. (Wright stain; original magnification, × 900.)

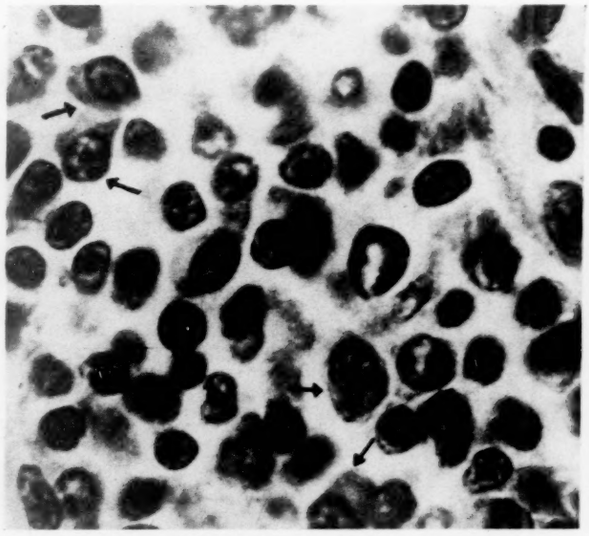

FIG. 7. Case VIII, V. H. Cervical lymph node. Typical mature plasma cells (arrows) are scattered among lymphocytes which form the predominant cell type. Lymphoid cells containing a rather abundant cytoplasm and showing some clumping of the chromatin probably represent transitional forms between lymphocytes and plasma cells. (Hematoxylin and eosin; original magnification, × 1000.)

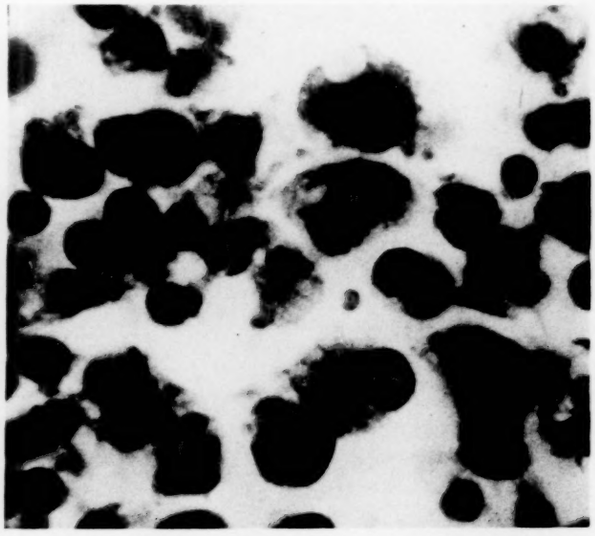

FIG. 8. Case X, S. K. Cervical lymph node. Plasma cells showing various stages of disintegration are intermingled with lymphocytes. Note the presence of intracytoplasmic vacuoles in several plasma cells. (Hematoxylin and eosin; original magnification, × 1000.)

CASE X. S. K. (F.D.H. 4421), a sixty-two year old white woman, had an eighteen month history of weakness and cervical and axillary lymphadenopathy. Physical examination on admission in June, 1954 disclosed generalized lymphadenopathy and hepatosplenomegaly.

Laboratory data revealed the following: Hemoglobin, 4.8 gm. per cent; white blood cells 16,500 per cu mm., lymphocytes, 73 per cent with approximately half the lymphocytes appearing immature and somewhat resembling plasma cells; serum protein, 7.7 gm. per cent. A markedly homogeneous abnormal protein was present in the serum (Fig. 1) and urine. X-rays of the skeleton revealed no abnormalities except for a partial collapse of the body of L-1. A biopsy specimen of a cervical lymph node showed a picture consistent with chronic lymphocytic leukemia, with

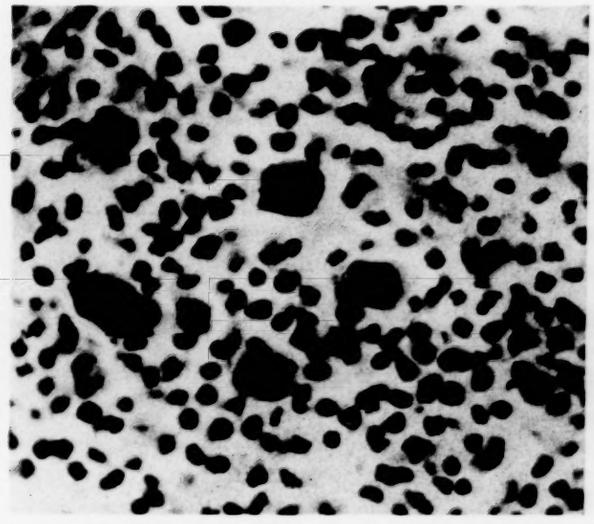

FIG. 9. Case XI, A. G. Hodgkin's granuloma in spleen. Five Dorothy Reed cells are illustrated. The plasma cells here are small and of the mature type. (Hematoxylin and eosin; original magnification, × 430.)

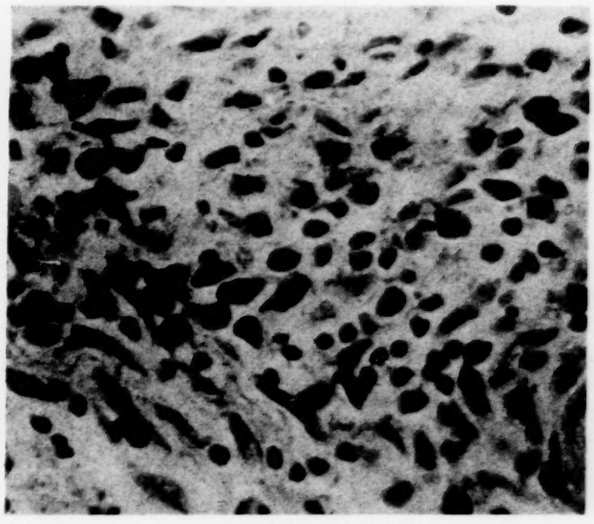

FIG. 10. Case XI, A. G. (Cf. Fig. 9.) Numerous mature plasma cells are intermingled with lymphocytes and fibroblasts. (Methyl green-pyronin Y; original magnification, × 430.)

the addition of a marked degree of plasmacytosis. The patient was treated with cortisone. Symptoms of congestive failure developed. In December, 1955, she suddenly became confused and died.

At autopsy, there was generalized enlargement of the lymph nodes most marked in the mediastinum, mesentery and retroperitoneum. The bone marrow was homogeneously red-brown and exuded freely from the cut surfaces of the ribs and vertebrae. A localized area of hemorrhage was present in the right cerebral hemisphere. The spleen weighed 1,800 gm. and the liver 2,600 gm. The heart was moderately hypertrophied. There was acute pulmonary congestion. Histologic examination of the lymph nodes revealed loss of the follicular pattern. The peripheral and medullary sinuses were filled with small lymphocytes. These also invaded the capsule. Plasma cells constituted 5 to 30 per cent of the total number of cells within lymph nodes. (Fig. 8.) There was mixed lymphocytic and plasmacytic infiltration of the bone marrow, spleen, liver, lungs and kidneys.

CASE XI. A. G. (F.D.H. 4655), a sixty year old white man, was admitted in November, 1954 with weakness, anorexia and weight loss of one year's duration. Physical examination revealed bilateral cervical lymphadenopathy and hepatosplenomegaly.

Laboratory data revealed the following: Hemoglobin, 6.2 gm. per cent; white blood cells, 16,500 per cu. mm.; neutrophils, 28 per cent; lymphocytes, 68 per cent; monocytes, 4 per cent, total protein, 5.4 gm. per cent. A discrete homogeneous protein with the mobility of a fast gamma globulin was seen on electrophoresis. (Fig. 1.) X-rays of the skeleton showed no evidence of disease. Biopsy specimen of a cervical lymph node was interpreted as Hodgkin's disease. The follicular pattern in this node was effaced. There were numerous reticulum cells, lymphocytes in various stages of maturity and Dorothy Reed cells. In some areas the lymphoid cells resembled mature plasma cells. Following a course of thio-tepa there was marked regression in the size of the enlarged lymph nodes but thrombocytopenia and leukopenia developed. Terminally, the patient became edematous and died in December, 1954.

At autopsy, the changes seen in various lymph nodes, spleen, liver, appendix and skeleton were still characteristic of Hodgkin's disease. (Fig. 9.) In addition, there were areas of fibrosis in several lymph nodes and marked hypoplasia of the bone marrow. These changes may have been due to the effects of chemotherapy. Mycotic abscesses were present in the lung. Plasma cells were present in great number in several tumor areas. (Fig. 10.)

This is the only case of Hodgkin's disease known to us in which an anomalous globulin component has been documented. Electrophoretic studies of the serums of patients with Hodgkin's disease have demonstrated non-specific changes such as hypoalbuminemia, a diffuse increase in gamma globulin or elevated alpha globulins [*12–15*], but myeloma type proteins have not been recorded. The concurrent plasmacytosis in this case may conceivably be responsible for the abnormal serum protein.

RESULTS

Cases Studied. In all thirteen cases studied the predominant histologic picture was that of proliferation of lymphoid cells and reticulum cells. Marked plasmacytosis (over 10 per cent) was observed in six of the thirteen cases (II, III, V,

TABLE II

PLASMACYTOSIS IN PATIENTS WITH MALIGNANT LYMPHOMA AND LEUKEMIA EXHIBITING MYELOMA-TYPE SERUM ABNORMALITIES

Case No.	Initial Diagnosis	Degree of Plasmacytosis	Remarks
I	Reticulum cell sarcoma	0*	Autopsy
II	Lymphosarcoma, lymphocytic type	+++	.....
III	Lymphosarcoma, lymphocytic type	+ − ++	.....
IV	Lymphosarcoma, lymphocytic type	0†	.....
V	Lymphosarcoma, lymphocytic type	± − +++	Autopsy
VI	Lymphosarcoma versus multiple myeloma	0†	Only bone marrow available for study
VII	Lymphosarcoma versus multiple myeloma	0†	Only bone marrow available for study
VIII	(?) Lymphosarcoma and paramyloidosis	+ − +++	.....
IX	Lymphocytic leukemia, subleukemic stage	±	Only bone marrow available for study
X	Chronic lymphocytic leukemia	+ − +++	Autopsy
XI	Hodgkin's disease	+ − ++	Autopsy
XII	Chronic lymphocytic leukemia	±	Only bone marrow and sections from small lymph node available for study
XIII	Chronic lymphocytic leukemia	±	Only bone marrow available for study

* No mature plasma cells present. Strongly pyroninophilic reticulum cells resemble myeloma cells.

† No mature cells present. The cytoplasm of the lymphocytes is more abundant than ordinarily seen and exhibits a strong pyroninophilia.

VIII, X and XI). In the remaining seven cases, one patient (Case I) showed strongly pyroninophilic reticulum cells resembling immature myeloma cells (Fig. 2), and in three instances (Cases IV, VI and VII) the majority of the lymphoid cells contained a rather abundant cytoplasm which also showed a strong affinity for pyronin (Fig. 5). The final group of three cases (IX, XII and XIII) is considered poorly documented as only bone marrow aspirations and, in one case, sections from a small lymph node were available for examination. In these three cases the histologic picture was that of a lymphosarcoma or lymphatic leukemia. There were no myeloma cells and the lymphoid cells presented no differentiating features such as abundant cytoplasm and pyroninophilia. The degree of plasmacytosis in all thirteen cases is listed in Table II.

Plasmacytosis, when present, varied moderately in degree in different sites and was usually demonstrated in most of the tissues which were involved by the disease process. Although small aggregates of plasma cells were frequently encountered, in only one case (V) were the majority of the infiltrating cells in one region of the body plasma cells. (Fig. 4.) Transitional forms between reticulum cells and lymphocytes on one hand, and plasma cells on the other, were frequently encountered. (Fig. 7.)

Control Cases. The electrophoretic findings and the degree of plasmacytosis in the twenty-five control cases are listed in Table III. The degree of plasmacytosis generally paralleled the estimated serum gamma globulin concentration, i.e., none of the twelve patients with hypogammaglobulinemia showed any appreciable degree of plasmacytosis in tissues. A marked plasmacytosis was observed in only one control patient (VI) with reticulum cell sarcoma. This patient's serum showed a diffuse hypergammaglobulinemia. In general, plasmacytosis, pyroninophilia and hypergammaglobulinemia were infrequent in this control group.

The methyl green-pyronin Y stain was noted to be very useful in this study. Pyronin has a strong affinity for ribonucleoprotein and stains the cytoplasm of plasma cells a deep red. Mast cells, some undifferentiated reticulum cells and lymphoid cells are also strongly pyroninophilic. The cytoplasm of histiocytes, most reticulum cells, lymphocytes and normoblasts does not show any particular affinity for pyronin. The chief value of the periodic acid-Schiff stain in this study was in differentiating megakaryocytes from Dorothy Reed cells. The cytoplasm of the megakaryocytes usually gives a strongly positive Schiff reaction in contrast to that of the Dorothy Reed cells. The Wilder reticulum stain helps considerably in differentiating reticulum cell sarcoma from an anaplastic carcinoma.

TABLE III

ELECTROPHORETIC FINDINGS AND DEGREE OF PLASMACYTOSIS IN THE CONTROL GROUP OF TWENTY-FIVE PATIENTS WITH LYMPHOMA AND LEUKEMIA NOT EXHIBITING MYELOMA-TYPE SERUM PROTEIN ABNORMALITIES IN WHOM AUTOPSIES WERE PERFORMED CONSECUTIVELY

Autopsy	Diagnosis	Electrophoretic Findings*					Degree of Plasmacytosis†
		γ	β	α_2	α_1	Albumin	
I. 721	Reticulum cell sarcoma	↙ +	N	↗ ++	↗ +	↙ +	0
II. 795	Lymphosarcoma	↙ +	N	↗ ++	↗ +	↙ ++	0
III. 818	Chronic lymphatic leukemia	N	N	N	N	N	0 − ±
IV. 878	Lymphosarcoma	N	N	↗ +	N	↙ +	± − ++
V. 895	Hodgkin's disease	N	N	↗ ++	N	↙ +	0 − ±
VI. 922	Reticulum cell sarcoma	↗ +++	↗ ++	↗ ++	↗ +	N	0 − +++
VII. 933	Reticulum cell sarcoma	↙ +	↗ +	↗ +	↗ +	↙ ++	0
VIII. 941	Chronic lymphatic leukemia	↙ +	N	↗ ++	↗ ++	↙ ++	0
IX. 959	Reticulum cell sarcoma	↙ +	N	↗ ++	↗ +	↙ +	0
X. 966	Reticulum cell sarcoma	N	N	N	N	N	0
XI. 975	Reticulum cell sarcoma	↗ +	N	↗ +	↗ +	↙ +	0
XII. 978	Chronic lymphatic leukemia	↙ ++	N	N	N	↙ ++	0
XIII. 983	Hodgkin's disease	N	N	↗ +	↗	↙ ++	0
XIV. 989	Lymphosarcoma	↙ +	N	↗ ++	↗ +	↙ +	0
XV. 991	Chronic lymphatic leukemia	↙ +	N	N	N	N	0
XVI. 997	Hodgkin's disease	↙ ++	N	↗ +	↗ +	↙ +	0 − ±
XVII. 1000	Reticulum cell sarcoma	N	N	↗ +++	N	↙ +	+
XVIII. 1006	Lymphosarcoma	↙ ++	N	N	N	↙ +	±
XIX. 1036	Chronic lymphatic leukemia	↗ +	N	↗ +	N	↙ ++	±
XX. 1044	Hodgkin's disease	↙ ++	N	N	N	↙ ++	0
XXI. 1060	Reticulum cell sarcoma	N	N	N	N	↙ +	0
XXII. 1070	Hodgkin's disease	N	N	N	N	N	0
XXIII. 1106	Lymphosarcoma	N	N	↗ +	N	N	0
XXIV. 1107	Reticulum cell sarcoma	↙ +	N	↗ +	N	↙ +	0
XXV. 1155	Chronic myelocytic leukemia	N	N	N	N	N	+

* N = normal. ↙ + = moderate decrease. ↙ ++ = marked decrease. ↗ + = moderate elevation. ↗ ++ = marked elevation. ↗ +++ = very marked elevation.

† 0 = plasma cells absent or rare. ± = 1–5%. + = 5–10%. ++ = 10–25%. +++ = 25–50%.

COMMENTS

The accumulation of plasma cells in the bone marrow and lymphoid tissues has been repeatedly associated with hyperglobulinemia. Bing and Plum [*1*] were the first to state clearly that "a common feature for the various affections in which hyperglobulinemia is a factor is an increase in plasma cells and cells belonging to the reticulo-endothelial system within and outside the bone-marrow; this seems to indicate that the globulin formation is connected with these cells." Plasma cells, on the other hand, are virtually absent in the bone marrow and lymph nodes of patients with agammaglobulinemia [*16*]. There is strong evidence that the abnormal proteins in multiple myeloma are products of the so-called myeloma cells [*17*].

Whether or not lymphocytes and reticulum cells, in the absence of plasma cells and their direct precursors, are capable of synthesizing globulin components is still a matter of conjecture. Kass [*18*] demonstrated the occurrence of normal serum gamma globulin in human lymphocytes. Dougherty, White and Chase [*19*] showed that transplanted non-metastasizing lymphosarcoma contained a high concentration of an immune globulin. Sundberg [*20*] pointed out that "we are still faced with the fact that, even with remarkable antibody production, plasma cells are often not as numerous as lymphocytes, and some type of lymphocytic reaction generally accompanies plasmacytosis."

The presence of intermediate forms among reticulum cells, lymphocytes and plasma cells has been noted in experimental studies [*21*].

Designations such as undifferentiated reticuloendothelial cells or lymphoid cells [*1,4*], plasma cell variants [*5*], pyroninophilic mesenchymal cells [*22*] have been used to indicate such transitional cells in man. Transitional forms have been observed in hyperplastic lymph nodes and in the predominantly lymphoid neoplasms in this study. It is possible that the pyroninophilic reticulum cells and large lymphoid cells encountered in our cases represent immature myeloma cells or plasma cells precursors. Pyroninophilia, although not a specific property of the plasma cell series, may in these instances indicate increased intracytoplasmic synthesis or storage of gamma globulin. These observations suggest that plasma cells may be forms of lymphocytes and reticulum cells in which qualitative or quantitative alterations in nucleoprotein metabolism have taken place.

The interrelationship of the various lymphatic neoplasms has been emphasized particularly by Custer [*23*] and Craver [*24*]. It remains to be stressed that intermediate forms exist and overlapping occurs between the lymphatic tumors and the plasmacytomas. In this study, ten of thirteen patients with malignant lymphoma and lymphatic leukemia, associated with abnormal serum proteins characteristic of multiple myeloma, showed a marked plasmacytosis or the presence of a large number of pyroninophilic reticulum cells and lymphoid cells. These findings indicate a cellular derivation and a potential metabolic disturbance common to some lymphatic neoplasms and the plasmacytomas.

SUMMARY

1. Ten of thirteen patients with malignant lymphoma and lymphatic leukemia, including one patient with Hodgkin's disease, all associated with abnormal serum proteins characteristic of multiple myeloma, showed a marked plasmacytosis or the presence of a large number of pyroninophilic reticulum cells and lymphoid cells in tissue sections and/or bone marrow aspirations. Intermediate forms between reticulum cells and lymphocytes on one hand, and plasma cells on the other, were frequently seen in the tissues studied.

2. Plasmacytosis and the presence of pyroninophilic reticulum cells and lymphoid cells were insignificant features in a control group of twenty-five patients with malignant lymphoma and leukemia, associated with normal or nonspecifically abnormal serum electrophoretic patterns, in whom autopsies were performed consecutively.

3. This study suggests, that clinically and morphologically, transitional forms occur, with considerable overlapping, between the various lymphatic tumors and the plasmacytomas.

Acknowledgment: We are grateful to Dr. Edith E. Sproul and Dr. Arthur Purdy Stout for their help, advice and interest in this work. We are also indebted to Mrs. Frances Roth and Miss Loretta Farrell for their technical assistance, and to Mr. Edward Hajjar for the photomicrographs.

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Review

Gastrointestinal Malabsorptive Syndromes*

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STATES of malabsorption imply metabolic inadequacy of the small intestinal mucosa. This may result from (1) inadequate biochemical preparation of materials for absorptive attention, (2) uncontrolled rate of delivery of nutrients to the small intestinal lumen, (3) lack of adequate contact time between nutrients and a normally absorbing intestinal surface, or (4) biochemical and structural defects of the intestinal mucosal cells. Occasionally also, there may be competition for use of ingested nutrients by bacteria under circumstances of unusual intestinal disease which allow intraluminal overgrowth.

Malabsorptive defects may be either specific as regards a single chemical compound, or broad and diffuse involving multiple nutrients. Striking examples of the former have been considered to be (1) pernicious anemia, wherein the stomach fails to produce intrinsic factor, a substance essential for conditioning of vitamin B_{12} for intestinal absorption, and (2) vitamin D-resistant osteomalacia, wherein the small intestinal mucosa appears insensitive to normal supplies of vitamin D, resulting in inadequate absorption of calcium. This interpretation of the latter condition has recently been questioned, for some studies now suggest that the primary metabolic defect may be in the bone itself [*189,195*]. This review will be concerned primarily with the more common malabsorptive syndromes wherein broad groups of nutritional elements are concerned.

The broad malabsorptive syndromes characteristically exhibit the most striking defects in the absorption of fat and fat-soluble substances. Excessive fecal fat loss, or steatorrhea, in the human adult is best defined as loss of greater than 5 per cent of fat ingested (coefficient of absorption less than 95 per cent [*1*] †). This may be put in terms of actual average daily loss while on a constant standard dietary intake. The standard diet of Wollaeger and associates [*2*] containing 101.6 gm. of daily fat, has been widely employed in the United States; normal subjects excrete less than 6.7 gm. of fat daily on this regimen (average 4.1 gm.).

† $\frac{\text{(Gm. ingested fat} - \text{gm. excreted fat)} \times 100}{\text{Gm. ingested fat}}$

BIOCHEMISTRY OF FAT DIGESTION AND ABSORPTION

The important processes involved in normal fat digestion and absorption are: (1) Efficient mechanical mixing of ingested fat with bile and pancreatic juices—fluids containing substances which biochemically condition lipid compounds for absorption by the small bowel. (2) Adequate emulsification of fats into small colloidal droplets, which thereby yields maximal surface for the action of lipase. Bile salts have long been recognized as important to this process; in addition, it is now known also that lipolysis products of triglycerides (monoglycerides, fatty acids) are helpful [*3,4*]. (3) Lipolytic action of lipase causing degradation of triglycerides to monoglycerides and free fatty acids. The most plentiful and efficient lipase is that from the pancreas. When the correct proportion of free fatty acid and monoglycerides in the presence of bile salts is reached, the remaining fat is spontaneously emulsified. The emulsion formed is stable at the normal intraduodenal pH during digestion. Its droplet size is small enough to allow efficient surface action of lipase or to permit direct particulate absorption. Provision of the necessary conditions for the formation of such emulsions may be a major function of pancreatic lipase. Relatively little is known about the lipase present in the exocrine secretion of the small intestine [*5,6*]; perhaps there is no lipase in the succus entericus itself, that identified intraluminally being extruded from the rapidly desquamating intestinal epithelial cells [*7,8*].

* From the Department of Medicine, University of Washington, Seattle, Washington. Supported in part by grants from the A. H. Robins Company, Richmond, Virginia and from funds for research in biology and medicine accruing through Initiative 171 of the State of Washington.

(4) Absorption of fat and its products by the surface mucosal cells of the small intestine—an extremely active biochemical process of great complexity. (5) Transport of absorbed lipids in collecting lacteals and portal venous capillaries. (6) Excretion of lipid by the gastrointestinal tract, usually considered negligible in amount.

Recent studies [*9*] indicate that fatty acids can be absorbed satisfactorily whether introduced into the intestinal lumen as glyceride or as free fatty acid. Intraluminal lipolysis of triglyceride has been shown to proceed in stepwise fashion successively through diglyceride and monoglyceride to fatty acid [*10–13*]. Proceeding in parallel with lipolysis, new ester-glyceride bonds may be formed, reincorporating free fatty acids into glycerides; however, the general direction of triglyceride hydrolysis proceeds toward eventual complete separation of fatty acid constituents [*10,14*]. Existing evidence indicates that either monoglycerides or free fatty acids are readily absorbed, but not di- or triglycerides [*12,15*]. The majority of absorbed fatty acids with chain lengths in excess of 10 carbon atoms are resynthesized in the small intestinal mucosal cells into new triglycerides [*12,15*]; in this form they are then routed via the intestinal lymph [*16,17*]. Fatty acids of shorter length are transported in the portal venous circuit as free fatty acids [*17,18*].

Intraluminar emulsification in the upper small intestine normally yields lipid particles of 0.5 microns or less in diameter [*3,19*]; an emulsifying system of bile salts, fatty acids and monoglycerides is appropriately efficient for triglycerides but not for paraffin oils [*19*]. However, pre-emulsified paraffin oil in particle diameter of 0.5 microns or less can be absorbed from the small intestinal lumen of rats [*19*]. This has led Frazer to suggest that any lipid which can be made into a sufficiently fine emulsion could directly cross the intestinal mucosal barrier without molecular alteration. This concept is supported by data concerning absorption of pre-emulsified lipids of varying particle size both in human patients with exocrine pancreatic insufficiency [*19*], and in normal rats [*20*]. The evidence for this theory of direct, particulate absorption is as yet meager as compared with the large amount of data demonstrating absorption of the chemical products of triglyceride hydrolysis.

Motility of the small intestine has long been considered to influence proportionately the rate of absorption of various nutrients. Accurate measurements to evaluate this belief have been exceedingly difficult to accomplish. This subject has recently been reviewed in brief by Cummins [*21*].

An irreducible amount of lipid is excreted in the feces, even during starvation, or while ingesting a fat-free diet [*22,23*]. Sterol excretion in bile and bacterial carcass lipid contribute minor portions; most of the endogenous excretion appears to be contributed by the small intestine [*24*], probably from its rapidly desquamating epithelium [*7,25*]; for example, in the adult rat, from 60 to 75 per cent of the ileal epithelium is desquamated daily [*7*], and for adult man it has been estimated that more than a half a pound of intestinal epithelial cells are extruded daily [*8*]. The question has been raised as to whether or not in steatorrheic conditions an increased excretion of endogenous fat may contribute importantly to the increased fecal fat. This has been unassessed in most of these disorders; in one case of celiac disease endogenous fat loss was increased by 2 to 5 gm. daily [*26*], but an increase was not found for one patient with non-tropical sprue [*27*]. In dogs, absence of bile or pancreatic juice from the intestinal tract causes a three fold increase in endogenous fat excretion; the total quantity remains small, however [*25*]. The chemical nature of the fat fed influences its degree of absorption. Defects in absorption are more likely to be observed if the dietary fat has a high proportion of saturated long-chain fatty acids, whereas unsaturated liquid fats are more readily absorbed [*25,28*].

The complete absence of bile or of pancreatic juice from the intestinal tract reduces fat absorption to 28 to 70 per cent of the ingested fat [*25,29–33*]. But noteworthy is the fact that considerable fat can be absorbed without the aid of these important digestive juices, even within isolated loops of small intestine [*34*].

DETECTION OF STEATORRHEA

Malabsorptive states vary widely in severity from conditions so mild as to defy easy recognition to those so catastrophically severe as to be obvious and life-threatening. Although diarrhea is usually present in marked chronic steatorrhea, it is occasionally absent [*35,36*]. At present, the three best methods of recognizing excessive fat loss seem to be the following: (1) Odor and gross appearance of stool: an experienced observer generally finds unmistakable the rancid

odor of excessive free fatty acids and silvery gray or creamy color which are characteristic of severe steatorrhea. (2) Microscopic stain for neutral fat and soaps: this simple procedure offers some security in confirming marked steatorrhea if carried out by experienced personnel, although a few investigators have recently questioned [*1*] this well established conviction. Regardless of the etiology of the steatorrhea, most of the fecal fat in the stool is present as soaps [*37–40*]. Therefore, even moderately severe steatorrheas can be missed by staining procedures if only staining for neutral fat is employed; hydrolysis with added glacial acetic acid and heat is routinely required for appraisal. This allows the droplets of melted, free fatty acid liberated from soaps also to stain with the lipid dye. (3) An intake-excretion study with accurate, quantitative chemical determination of daily fat loss while on a constant standard diet: There seems as yet no substitute for this intake-excretion measurement (a) to detect mild degrees of steatorrhea and (b) to quantitate the degree of fat loss. Moreover, this is by far the most reliable available technic for proving the presence of any steatorrhea [*36*]. The recent quantitative method for analysis of fecal fat by van de Kamer [*41*] has greatly simplified the laboratory aspects of this approach. Since states of malabsorption characteristically undergo spontaneous fluctuation in intensity, and bowel habits are often irregular and unpredictable, the more prolonged the period of fecal collections the more accurate is the appraisal of the patient's defect; a minimum of three days is usually required, and most investigators prefer four to twelve day duplicate collections [*37*].

Popular procedures, the diagnostic accuracy of which has been questioned, are (1) determination of per cent lipid in dry or wet single fecal specimens [*1*] and (2) the vitamin A "tolerance" test [*1,42*]. The first of these has been proved so unreliable that it should be entirely abandoned. Such "absorption tests" as that for vitamin A are designed to measure the rate and not the total quantity of an orally administered substance absorbed, and are unpredictably affected by variations in gastric emptying, distribution among bodily fluid compartments, metabolism and storage, and release from cellular depots.

Newer procedures which promise to be as useful in detecting or quantitating various malabsorptive defects include: (1) determination of radioiodine in two to three day fecal collections following an oral test meal of I^{131}-labeled triolein [*43,44*], (2) determination of fecal radioiron following an orally given dose [*45*], (3) determination of urinary, fecal or hepatic radiocobalt after an oral tracer dose of radiocobalt-labeled vitamin B_{12} given with and without intrinsic factor [*46–48*], and (4) quantitation of d-xylose in a five-hour urinary collection following oral administration of a test dose of this pentose [*49–51,196*]. Further data for each of these procedures are required for reliable appraisal.

CLASSIFICATION OF STEATORRHEAS

The following classification of the steatorrheas is based upon the principal points of the normal biochemical processes involved in fat digestion and absorption:

I. Inadequate mixing of food with bile salts, lipase
   1. Pyloroplasty
   2. Subtotal gastrectomy
   3. Total gastrectomy

II. Inadequate lipolysis—lack of lipase
   1. Pancreatic insufficiency
      - Congenital cystic fibrosis of pancreas
      - Chronic pancreatitis
      - Cancer of pancreas or ampulla
      - Pancreatic fistula
      - Protein deficiency

III. Inadequate emulsification of fat—lack of bile salts
   1. Obstructive jaundice
   2. Severe liver disease

IV. Primary absorptive defect of small bowel
   1. Inadequate length of normal surface
      - Surgical resection
      - Internal fistula
   2. Obstruction of mesenteric lymphatics
      - Lymphoma
      - Carcinoma
      - Whipple's disease
   3. Inadequate absorbing surface due to extensive mucosal disease
      - Inflammatory—tuberculosis, regional enteritis
      - Neoplastic
      - Amyloid infiltration
      - Scleroderma
   4. Biochemical dysfunction of mucosal cells
      - Celiac disease

Sprue
Severe starvation
Transient dysfunction associated with intestinal infections

5. Malabsorption associated with blind loops, diverticula, strictures

Although this classification has proved useful as a working basis for clinical differentiation of these groups, it is far too simplified from the standpoint of accuracy in biochemical etiology. Many conditions listed participate in more than one of these categories of biochemical derangement. A somewhat different but equally useful classification has been given by Frazer [*52*]. Adlersberg and Schein [*57*] have divided the malabsorptive syndromes due to small intestinal dysfunction into "primary" and "secondary" sprue syndromes, a useful concept; the latter term would include groups 1, 2, 3 and 5 of division IV of our classification. A very brief discussion of many of these conditions will be attempted.

Gastrectomy. The malabsorptive defects following gastric surgery are complex and not thoroughly understood. Excellent reviews have recently been published by Everson [*53*] and by Moore and Harkins [*54*]. Of special interest is the excessive loss of nitrogen as well as of fat following even partial gastrectomy [*53–56*]. Rearrangements of anatomic continuity, destruction of the gastric reservoir through resection, and ablation of the pyloric sphincteric mechanism are responsible for destroying the efficiency of mixing food with digestive juices.

Following total gastrectomy, fecal caloric losses are characteristically severe, usually greater than are observed following subtotal gastrectomy [*53,58,59*]. Following total gastrectomy, esophagoduodenal connection has resulted in less fecal nitrogen loss than esophagojejunal anastomosis [*60*] but has shown no difference in fecal fat loss [*58*]; some surgeons have therefore advocated esophagoduodenal connection, with interposed colon or a jejunal pouch if necessary, to provide improved nutritional status [*61,192*]. Following partial gastrectomy, gastroduodenal anastomosis has clearly been shown to result in less fecal fat and nitrogen loss than gastrojejunal connections [*58,62,63*]. However, gastroduodenal continuity does not necessarily prevent such nutritional losses [*54,56*] as illustrated by our study of such a patient. (Fig. 1.)

The presence of food and the products of its early digestion in the upper intestine is of marked importance in causing physiologic flow of bile and pancreatic juice through local release of cholecystokinin, secretin and pancreozymin. Following gastric surgery too brief a period of contact between the food bolus and the upper intestinal mucosa could easily result in suboptimal production of these digestive hormones. Furthermore, uninhibted forward progress of the ingested meal would logically result in decreased opportunity for thorough mixing with bile and pancreatic juices if these fluids were not immediately released. Obviously, anastomoses not retaining the duodenum in continuity would exaggerate such problems. In spite of these considerations, the administration of supplements of commercial bile salts and/or pancreatic extracts to such patients has largely been disappointing. (Fig. 1.) However, a few investigators have published intake-excretion studies indicating that the well timed administration of extremely large amounts of pancreatin [*53,64*] or of certain kinds of bile salts [*65*] may yield improvement in fat absorption. It should further be noted that at the dosage required for this, most bile salt preparations produce gastrointestinal disturbances [*65*].

Pancreatic Insufficiency. Exocrine insufficiency of the pancreas occurs in all degrees. Since this organ has a large reserve capacity, important nutritional defects do not result until its exocrine activity is almost completely missing. Thus, even diffuse disease of this organ may be present without absence of duodenal enzymes and absorptive defect [*66–68*]. Even after removal of the head of the gland or complete surgical extirpation of it in adult human subjects, a large share of dietary nitrogen and fat can be absorbed, even if partial gastrectomy also has been performed [*32,69,70*]. Fecal losses of fat and nitrogen in achylia pancreatica [*31,32*] have been found to be no greater than in other types of malabsorptive syndromes herein discussed.

The enzyme content of exocrine pancreatic secretion is probably very sensitively influenced by the dietary protein intake. Normally, the daily quantities of enzyme protein manufactured are enormous, and the rate of uptake of labeled amino acids by the acinar cells is very rapid [*71*]. It is not surprising, then, that protein malnutrition has been shown to suppress pancreatic enzyme formation [*72,73*]. Extreme protein lack, as in kwashiorkor, eventually results in necrosis and atrophy of the acinar cells [*72,73*]. Similarly,

STEATORRHEA FOLLOWING SUBTOTAL GASTRECTOMY

H.A. – Billroth I

PERIOD # (5 DAY)	REGIMEN (GM./DAY)	DIET FAT (GM./DAY)	STOOL FAT (GM./DAY)	DIET PROTEIN (GM./DAY)	STOOL N (GM./DAY)
I	Control	100	26.1	118	
II	Control	100	26.0	118	3.0
III	Tween 80, 7.5gm.*	100	20.3	118	3.0
IV	Tween 80, 7.5gm.*	100	18.5	118	2.87
V	Lipomul, 44gm.**	100	22.7	118	3.28
VI	Lipomul, 44gm.**	100	21.8	118	3.11
VII	Control	100	25.5	118	3.12
VIII	Pancreatin B, 9gm.	100	27.3	118	
IX	Pancreatin B, 9gm.	100	26.8	118	
X	High fat	185	33.2		
XI	High fat	185	49.2		
Normal reference (2)		101.6	4.1 ± 2.6	117.5	1.7±0.8

* Furnished by Abbott Laboratories

** Furnished by Dr. E. L. Burbidge, The Upjohn Company; particle diameter of peanut oil droplets was presumed to be 0.5 to 1.0 microns.

Pancreatin B: Panteric Capsules ("triple strength"), Parke, Davis & Company.

FIG. 1. Fat and nitrogen intake-excretion studies on a fifty-eight year old woman who six months previously had undergone a 70 per cent gastrectomy with gastroduodenostomy (Billroth I) for benign duodenal ulcer. Secretin test and x-ray examinations of the small bowel were normal. The study demonstrates excessive fecal loss of both nitrogen and fat, increased total absorption of fat on higher fat diet (Periods X, XI), and non-effectiveness in improving fat absorption of (1) emulsifying agent (Tween 80), (2) pancreatic extract, and (3) pre-emulsification of a large proportion of the dietary fat (lipomul®). This study was conducted with Dr. Ian R. Mackay..

interference with the gland's rapid amino acid metabolism by administering ethionine has resulted in exocrine insufficiency from acinar cell necrosis [*74,75*]. In addition, a lack of specific amino acids in the lumen of the upper small intestine may prevent adequate release of pancreozymin, which is necessary to stimulate pancreatic enzyme production [*76,77*].

Hepatobiliary Disease. The occurrence of steatorrhea during obstructive jaundice has long been recognized [*78*]. Lack of bile salts in the intestinal lumen results in malabsorption of all lipoid compounds, including fat-soluble vitamins such as vitamin K; thus, for years, surgeons have emphasized the importance of administering vitamin K-active compounds routinely to jaundiced patients being prepared for surgery. In long-standing biliary cirrhosis, steatorrheic losses of vitamin D and calcium may cause osteomalacia [*79,191*]. That reduction in such losses of fecal fat may be brought about by returning the patient's own bile to his intestinal tract is accepted. However, little can be accomplished in this regard by giving commercial bile salt preparations unless very large quantities are administered [*52,80*]; even in huge quantities, some bile salt compounds have been shown to be completely ineffective [*80*]. Since synthesis of bile salts by the liver is easily suppressed by hepatic injury [*81–83*], it would naturally be expected that resultant steatorrhea would be found in many cases of severe primary liver disease, whether acute or chronic. Fat intake-excretion studies by Gross and associates [*84*] have clearly demonstrated that this occurs.

Disorders of the Small Intestine. It is obvious that the absorptive surface of the small bowel is essential to life. There appears to be marked

individual variation in its reserve capacity. In reviewing the data concerning nutritional defects following extensive resections of this organ, it is apparent that wide variations in individual response occur [*85,86*]. Absorption is not usually impaired if less than 50 per cent is removed. Occasionally, very short remaining lengths (3 to 7 feet) of normal upper small bowel can suffice for maintenance of adequate nutrition [*87–89*]. The remaining remnant of normal small bowel may undergo hypertrophy and progressively increase its efficiency [*85*]. There has been much controversy as to whether or not the site for absorption of specific nutrients may be localized in definite anatomic regions of the small intestine; the evidence to date appears conflicting and inconclusive. After resection for inflammatory disease (such as regional enteritis), the efficiency of function of the remaining intestine to some extent depends on whether or not active disease remains. Following extensive removal, fat utilization is poorest, protein absorption less impaired, and carbohydrate absorption little, if any, affected [*90*]. Calcium deficiency is frequently noted. This has been thought to be due partly to inabilty to reabsorb the calcium from the digestive secretions [*88*]. An increase of dietary fat increases such fecal calcium loss [*91*], as is also the case in sprue [*27*], and this loss is probably the result of increased formation of insoluble calcium soaps in the lower intestine. The published studies of nutritional losses following extensive resection of small bowel have recently been compiled by Pietz [*92*].

Occlusion of major mesenteric lymphatics by involvement of the lymph glands with lymphoma, tuberculosis or Whipple's disease may give rise to the steatorrheic syndrome [*93*]. Similarly, occlusion of the cisterna chyli by direct extension of pancreatic cancer or lymphoma may obstruct lacteal drainage and result in steatorrhea. Probably the malabsorption syndromes associated with Whipple's disease should not be considered purely obstructive in mechanism; the dramatic remissions effected by steroid hormones [*94–96*], and histochemical studies [*94,97,98*], suggest that biochemical dysfunction of small intestinal mucosal cells may be primary.

Celiac disease in childhood, adult sprue, starvation steatorrhea and pellagrous steatorrhea all seem to represent local biochemical disorders of the small bowel epithelium. There has been a long controversey as to whether structural alteration of the small intestinal epithelium may precede or accompany the severe dysfunction. The autopsy material for sprue described in the past has demonstrated little histologic abnormality of the small bowel mucosa [*99,100*]. The occurrence of rapid autolysis of this organ postmortem has caused doubt as to the conclusiveness of these reports [*100–102*]. Fresh biopsy or cytologic material from the small intestine of patients with sprue has been extremely scarce [*102*]. The recent development of duodenal biopsy instruments [*103*] allows new opportunities to collect specimens for study by the newer morphologic technics. The small intestinal epithelium, which is responsible for so much daily metabolic activity and is capable of so rapid a rate of protein synthesis [*104*] and renewal of its cells [*7,105*], should readily become incapacitated by severe malnutrition; indeed, intestinal mucosal atrophy and the sprue syndrome are well known features of advanced starvation and kwashiorkor [*72*]. Paulley [*102*] has reported, in his biopsy specimens from patients with sprue, thinning of the mucosa, clubbing and edema of the villi, and infiltration of plasma cells and eosinophils. These abnormalities are somewhat reminiscent of the more severe changes caused by folic acid antagonists [*106–108*]. Presumably, pteridine deficiency would greatly interfere with renewal of the small bowel epithelial cells, and one can speculate that eventually such damage would become irreversible.

Recently a tentative subdivision of these sprue syndromes has been attempted [*52*], based upon response to various therapeutic regimens. These include (1) folic or folinic acid therapy, dramatic response to which suggests a specific nutritional deficiency [*99,100,109*]; (2) gluten-free diet; (3) treatment with antibiotics, suggesting overgrowth of luminal bacteria as partially etiologic [*52,190*]. Of interest is the characteristic spontaneous fluctuation in severity of the biochemical lesions in the constitutional celiac or "nontropical" sprue syndrome. Pregnancy, infection and malnutrition characteristically precipitate new exacerbations. There is a striking occurrence of sprue in adult life in patients known to have had the celiac syndrome in childhood [*100,110*]. Excellent comprehensive reviews of the celiac and sprue syndromes have recently appeared [*100,111,112*].

Broad malabsorptive defects are occasionally associated with small intestinal strictures, blind loops and multiple diverticula. Vitamin B defi-

ciencies and megaloblastic anemias are common in these situations, and are presumed to be due to overgrowth of luminal bacteria in such sites of intestinal stasis [*113,114*]. However, malabsorption of fat and other nutrients is also well documented in a few carefully studied cases, the explanations for which remain more mysterious [*36,52,115–117*]; for this phenomenon, an overgrowth of bacteria has likewise been postulated, since some cases have appeared to respond to antibiotics.

DIFFERENTIATION OF PANCREATIC FROM ENTERIC STEATORRHEA

Frequently difficult is the differentiation between malabsorptive syndromes due to pancreatic insufficiency and those caused by a primary disease in the small bowel. In the adult, exocrine pancreatic insufficiency is usually the result of chronic recurring pancreatitis; cancer as a cause is far less common. A diagnosis of carcinoma usually requires cytologic or histologic identification. Although it has been believed that surgical laparotomy is usually required to establish a diagnosis of carcinoma of the pancreas during life, recent work [*118,119,198*] has proved that careful cytologic examination of duodenal contents, especially following secretin injection [*119,198*], may yield a positive diagnosis in 50 to 60 per cent of cases. Even at laparotomy, differentiation from chronic pancreatitis may be difficult; surgical biopsy of a firm pancreas may reveal only the pancreatitis surrounding a carcinoma, and presents considerable risk of pancreatic fistula. Chronic pancreatitis is suggested by a characteristic long history of recurring attacks of upper abdominal pain [*120,121*]; however, this destructive disease may run its full course painlessly [*122*]. Rarely, pancreatic disease is detected by palpating a pancreatic cyst or tumor. Calcification in the pancreas, if observed by roentgen abdominal survey film, clearly identifies chronic pancreatitis [*123,124*]; however, this disease may be present in an advanced state even in the absence of this diagnostic finding [*120,124*]. The presence of mild diabetes mellitus accompanying steatorrhea suggests simultaneous destruction of both pancreatic islets and exocrine tissue. However, it has been found that *either* insulin production or exocrine secretion may separately become insufficient as the result of chronic pancreatitis [*125*].

Proof that inadequacy of the external secretion of the pancreas causes a given malabsorption syndrome depends upon laboratory studies demonstrating one or more of the following: (1) virtual absence of proteolytic and lipolytic enzymes in the duodenal juice, (2) absence of pancreatic response to intravenous secretin, (3) clear-cut improvement in absorption of dietary fat and nitrogen by administering pancreatin, as demonstrated by quantitative intake-excretion studies.

The gelatin film test [*126*] for proteolytic activity in stools or duodenal juice has gained popularity as a convenient screening test for pancreatic insufficiency; it is, however, not of great relability for duodenal contents [*127*] and is of value for stool specimens only in early infancy [*128*]. Therefore, duodenal juice should be examined for enzymatic activity in the laboratory by more standard procedures. Shwachman [*66*] has emphasized that patients with diffuse pancreatic disease may have very small amounts of digestive enzymes in their pancreatic secretion despite steatorrhea and azotorrhea resulting from a marked decrease in total enzyme production. Lack of response to secretin in increased volume, bicarbonate concentration and total bicarbonate of pancreatic juice readily supports a diagnosis of pancreatic insufficiency [*129,130*]. Intake-excretion balances of fat and nitrogen to test the efficacy or oral pancreatin require extensive effort. Special problems encountered include (1) choice of commercial pancreatic extract (potency varies widely, and some preparations are ineffectual [*31*]) and (2) choice of dose to be employed; an important example of the latter problem is presented in Figure 2. Since all degrees of diminution in output of pancreatic enzymes are encountered, some patients may be aided effectively by relatively small quantities of pancreatin (5 to 8 gm. daily), whereas complete absence of exocrine function generally necessitates the giving of very large amounts (15 to 30 gm. daily) [*31*]. In this laboratory it has been observed that a decrease in number of daily bowel movements and cessation of diarrhea cannot, in adults, be relied upon as proof that pancreatin has been effective in diminishing fat and nitrogen losses. In fact, we have noted poor correlation of number and volume of daily stools with results of quantitative chemical analysis of stool nitrogen and fat, an example of which is shown in Figure 2. It is now recognized that the determination of the ratio of split to unsplit fat is of no value in differentiating

pancreatic insufficiency from other types of steatorrhea [*37–40,131*]. The excellent review of Dreiling and Janowitz [*132*] describes in some detail the difficulties encountered in establishing a diagnosis of exocrine pancreatic insufficiency by laboratory tests.

DIFFERENTIATION BETWEEN DISEASES OF THE SMALL INTESTINE

Differentiation between the "biochemical disorders" of the small intestine (sprue, celiac disease) and frank inflammatory or neoplastic diseases is usually accomplished by roentgen examination with barium suspensions. However, extensive involvement with lymphoma may occur without roentgenographic evidence [*133*]; similarly, the intestinal roentgen observations in cases of Whipple's disease are generally nonspecific [*95,134*]. Thus surgical exploration with biopsy may occasionally be required to identify intestinal lymphoma, amyloid infiltration, tuberculous enteritis or Whipple's disease. Recently, the importance of a diagnostic search for extraintestinal manifestations of Whipple's disease has been recognized. The findings of peripheral or mediastinal lymphadenopathy and sacroiliac arthritis have been helpful, and the diagnosis has been made by peripheral lymph node biopsy [*97,134*]. The development of chylous ascites in association with steatorrhea indicates inflammatory or, more commonly, neoplastic obstruction of the cisterna chyli or of the collecting lacteals of the mesentery. Although cytologic examination of such peritoneal fluid can occasionally identify carcinoma, surgical exploration with direct biopsy is generally required for certain diagnosis.

PATHOPHYSIOLOGY OF CLINICAL SIGNS AND SYMPTOMS

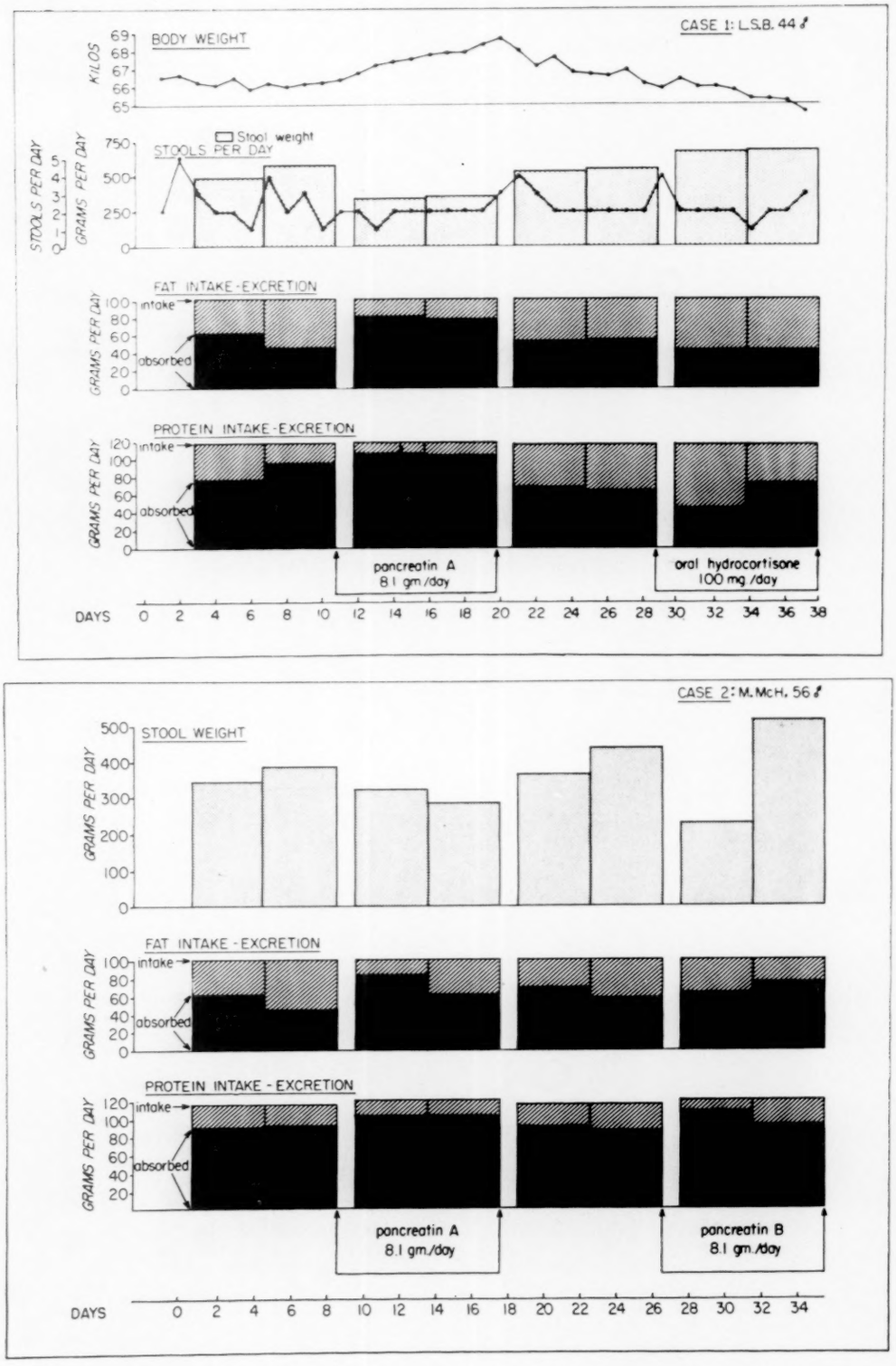
Clinical manifestations of the broad malabsorptive syndromes and the presenting complaints of such patients are highly variable [*135,136*]. The characteristic pathophysiology of the development of the various clinical signs and symptoms is depicted in Figure 3. It will be noted in this diagram that at least twelve different problems may present themselves: hemorrhagic phenomena, diarrhea, tetany, osteomalacia and/or osteoporosis, general malnutrition, edema, amenorrhea, megaloblastic or iron-lack anemia, glossitis and cheilosis, and peripheral neuritis.

Steatorrhea is always present during exacerbations. As the absorptive defect increases in severity, simple water-soluble compounds may become poorly absorbed, and manifestations of their lack thus appear. Great variation in the selectivity of such defects has been found [*137*]. In fulminant sprue there may appear to be almost no absorption of nutrients ("intestinal shut-down"); even ingested water is retained in the lumen along with unabsorbed osmotically-active compounds [*138*].

Of special interest are recent investigations concerning the roentgen barium pattern of the small intestine in the various steatorrheas. The classic changes of this so-called "deficiency pattern" have been described many times [*100*], and have recently been reviewed comprehensively by Aldersberg and associates [*139*]. These include dilatation, segmentation and scattering of barium suspensions, abnormal motility, apparent thickening of folds, moulage sign and an increase in intraluminal fluid. French, Ardran and their colleagues have demonstrated [*140–142*] that many of these well known abnormalities are presumably caused by precipitation of the colloidal barium sulfate by intraluminal contents, especially the excessive mucus produced by the stimulus of increased fatty acids. Chemical abnormalities of the gastric and small intestinal mucus in sprue have been discovered [*143*], and these may also influence flocculation of barium sulfate suspensions. In both steatorrheic and normal patients, normal or "deficiency" patterns have been produced at will, depending upon the type of barium suspension employed. In our experience, the "deficiency pattern" does not correlate well with the degree of steatorrhea, nor is it diagnostic of any particular variety of malabsorptive condition.

To be emphasized is the marked variation in intestinal motility and rate of absorption of water in these cases. Diarrhea is not an essential feature of the steatorrheic syndrome, and does not correlate well with degrees of nutrient loss [*35,190*]; such patients may pass 50 per cent or more of their daily fat intake in a single daily semiformed stool. However, in acute exacerbations of sprue and other steatorrheas, diarrhea is most regular.

Probably the most serious single feature of the broad malabsorption syndromes is the simple loss of calories. Since the adult ordinarily depends primarily upon his fat intake for caloric supply, the selectively greater defect in fat absorption will usually manifest itself in



NOTE: Pancreatin A: Viokase tablets furnished by Dr. Ezra Levin, Viobin Corp.
Pancreatin B: Panteric capsules ("triple strength"), Parke, Davis & Co.

FIG. 2. See legend opposite page.

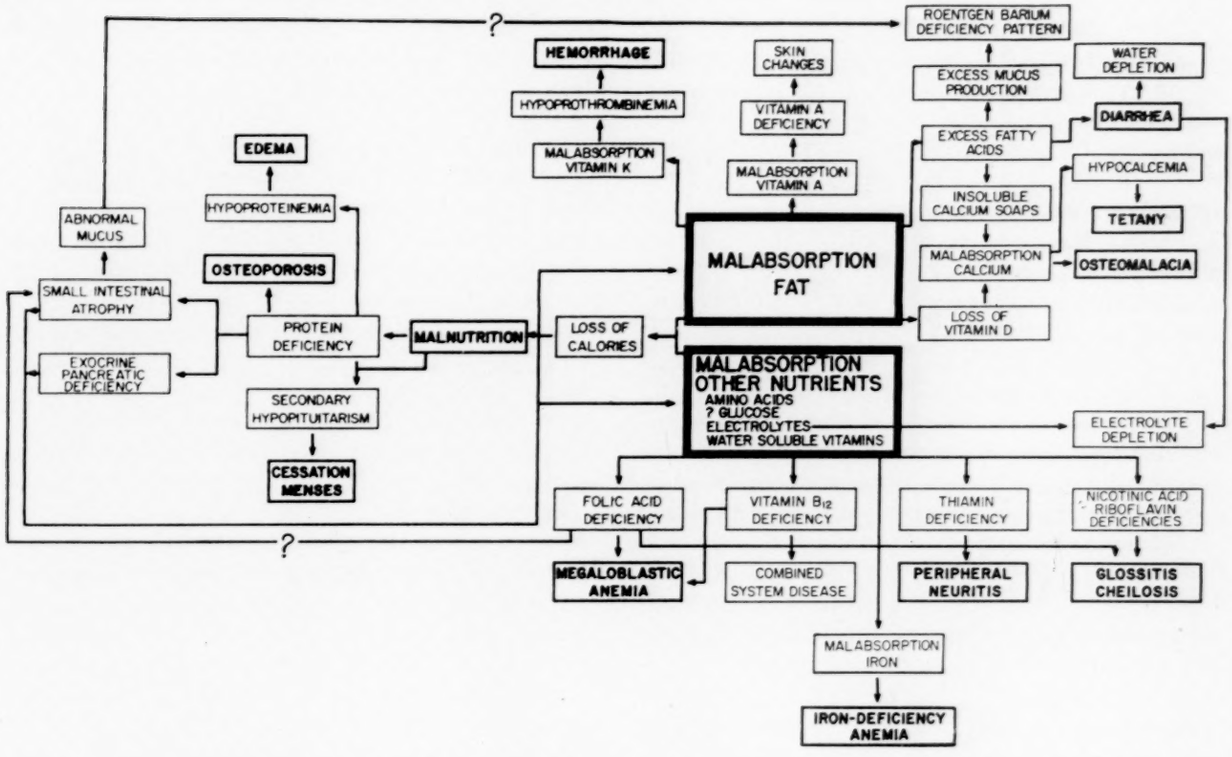


FIG. 3. Pathophysiology of the major signs and symptoms resulting from small intestinal malabsorption. The various typical presenting symptoms and findings are recorded in heavy lettering.

progressive weight loss and general malnutrition. As body protein stores are raided for the amino acids then required to provide essential calories through gluconeogenesis, additional malfunction of small intestinal mucosal and pancreatic acinar cells may result [*72,73,144*]. Inadequacy of amino acids for normal synthesis of polypeptide hormones may produce secondary hypopituitarism with resultant cessation of menses in young females.

The metabolic bone disorder resulting from severe prolonged steatorrhea is usually a mixed bone lesion. Disorders both in (1) formation of bone matrix from protein depletion (osteoporosis) and (2) demineralization from the long-standing negative calcium balance (osteo-

FIG. 2. Fat and nitrogen intake-excretion studies on two patients with pancreatic insufficiency, demonstrating varied response to pancreatic extract and difficulties in employing such balance studies to support the diagnosis. CASE 1 (studied with Dr. Beach Barrett): Long-standing chronic alcoholic with severe broad-deficiency syndrome. Long history of recurring prolonged episodes of epigastric pain. Identification of tuberculosis and onset of diarrhea occurred in 1950. In 1951 had 2/3 distal gastrectomy with gastroduodenostomy (Billroth I) and vagotomy for bleeding duodenal ulcer. At that operation active tuberculosis of liver, mesenteric lymph glands and small intestine was noted. Patient has since been continuously on antituberculous drug therapy. At time of this study pancreatic calcification was not present in roentgen films. X-ray study of the small bowel revealed no abnormality. Liver function was normal. Alkaline duodenal juice showed no proteolytic activity; there was no response to the standard secretin test. Pancreatin in relatively small dosage elicited a satisfactory response, both clinically as regards cessation of diarrhea and gain of weight, and laboratory-wise in improved absorption of fat and nitrogen. Hydrocortisone, as expected, was without similar efficacy. Subtotal gastrectomy and old, tuberculous obstruction of mesenteric lymphatics may have contributed to malabsorptive syndrome. Mild diabetes mellitus developed later. CASE 2 (studied with Drs. Francis Wood, Jr. and John Lee): Long-standing chronic alcoholic with severe diarrhea and malnutrition. No history of recurring painful abdominal episodes. Mild diabetes had been present since 1951. Roentgen studies showed normal small intestinal pattern; there was diffuse pancreatic calcification. Liver function was normal. Alkaline duodenal juice showed no proteolytic activity, and the standard secretin test showed non-response. Diarrhea ceased promptly with administration of the first pancreatin supplements, and one or two semi-formed bowel movements were passed daily thereafter in spite of complete lack of parallel improvement in the absorption of fat and nitrogen or in average daily stool weight. The dosage of pancreatin used in this case is believed to have been inadequate.

malacia) can be expected to be present. At times, the latter type may occur without symptomatic hypocalcemia (tetany); presumably a low concentration of ionized serum calcium levels is prevented by compensatory increased parathyroid activity. It has been suggested that such secondary hyperparathyroidism may further increase bone demineralization [*145*]. Although commonly recognized as a threatening feature of chronic sprue, osteomalacia may result from any variety of severe continuing steatorrhea; for example, it has even followed subtotal gastrectomy for benign peptic ulcer [*146*].

The hematologic defects of these malabsorptive disorders are protean. In typical sprue or celĭac disease, iron-deficiency anemia and megaloblastic anemia may be observed singly or in combination [*100,147*]; iron, folic acid and vitamin B_{12} are all poorly absorbed [*45,147*]. Megaloblastic anemia due to vitamin B_{12} malabsorption can regularly be expected to develop after total gastrectomy, due to absence of intrinsic factor [*48*], but does not result from partial gastrectomy unless gastric mucosal atrophy also occurs [*148*]. Folic acid or vitamin B_{12} deficiency may arise from a variety of small intestinal disorders (blind loops, diverticula, strictures, etc.) in which an abnormal overgrowth of intestinal bacteria competes with the host for these ingested compounds [*114*]. Although iron-deficiency anemias have been reported to follow gastric operations, opinion concerning the efficiency of absorption of iron under these circumstances is conflicting [*54,149*]. In our experience, iron-deficiency anemia, if present in the partially gastrectomized patient, usually responds very satisfactorily to oral iron supplements.

IMPORTANT ACCESSORY LABORATORY INVESTIGATIONS

All patients with recognized gross malabsorption syndromes should have certain routine laboratory and roentgen examinations. If hematocrit and appraisal by blood smear of the erythrocytes indicates anemia, bone marrow examination for determining state of iron stores [*150*] and megaloblastic activity is warranted. Low plasma prothrombin concentration even in the absence of ecchymosis may suggest inadequacy of absorption of vitamin K; prompt response from low to normal values after injection of menadione is confirmatory. Serum total protein and albumin concentrations are important in appraising the degree of protein malnutrition and the capacity of the body to maintain the homeostatic mechanisms supporting the plasma protein levels; a selective fall in serum albumin is characteristic of starvation syndromes. Determination of serum calcium, phosphorus and alkaline phosphatase are indicated in steatorrheic syndromes of prolonged duration to discover if hypocalcemia or osteomalacia is present. The adequacy of the ionized serum calcium concentration must be determined by a comparison of the total serum calcium with the serum total protein and albumin concentrations [*151,152*]. Osteomalacia, as evidenced by an increased serum alkaline phosphatase activity, may develop despite a normal serum calcium concentration. This situation has been attributed to response of the parathyroids to a previously decreased serum calcium. A low serum phosphorus concentration, which is often present, may reflect decreased renal tubular reabsorption of phosphate attributable to parathyroid overactivity [*197*]. Inadequate absorption of phosphorus from the intestine also may occur. A urine Sulkowitch test, or twenty-four-hour urine calcium excretion, provides a gross reflection of the serum ionized calcium concentration, and is therefore very useful as a measure of hypocalcemic tendency or an indication of overdosage with vitamin D. We have observed an occasional striking exception to this generalization in patients repairing severe osteomalacia, wherein urine Sulkowitch tests remained persistently negative for months following return to normal of the low serum calcium. Presumably this indicates a strongly positive calcium balance occurring during bone remineralization. An oral glucose tolerance test has traditionally been considered to indicate the degree of severity of a small bowel malabsorptive syndrome, failure to absorb simple, water-soluble compounds being reflected in a flat blood glucose curve; however, recently, the reliability of this test has been questioned [*50,153,188*]. Glucose tolerance tests would, of course, be of little aid in studying malabsorption after gastrectomy since alimentary hyperglycemia is characteristic. An oral vitamin A absorption test usually seems superfluous; if significant steatorrhea has already been identified, this test would confirm the obvious. Although studies by Legerton and associates [*154*] have demonstrated in some sprue patients a striking parallelism between the blood con-

centration of vitamin A five hours after an oral test dose and the degree of steatorrhea as defined by fat balance determination, the reliability of this test has been questioned by others [*1,42*]. If available, determination of per cent absorption of radiocobalt-labeled vitamin B_{12}, radioiron and folic acid [*147*] may be helpful in understanding the scope of the absorptive defect and providing more specific therapeutic implications.

Roentgen survey of the abdomen for calcification in the pancreas is indicated. In all steatorrheas roentgen studies of the small intestine with barium suspensions are needed. If the gross steatorrhea has been of longer than one year's duration, a roentgen survey of the spine, pelvis and chest for bony rarefaction should be made.

PRINCIPLES OF THERAPY

Treatment of the diffuse malabsorptive syndromes may be considered in two divisions, (1) general recommendations, and (2) therapy for specific varieties. General support and rehabilitation of bodily nutrition is the cornerstone to treatment. In severe cachexia prolonged parenteral supplementation may be required to supply both calories and amino acids. The oral dietary recommendations allow considerable variation in daily fat intake. Since it has been demonstrated [*2,33,131,137*] that fecal fat loss from mixed dietary fats is usually proportionate to total oral intake, a larger amount of dietary fat yields greater net absorption of fat than would a low fat intake [*155,156*]. The added calories may be of advantage despite increased steatorrhea [*157*]. The level of dietary fat must, however, be tailored to the individual patient, for the majority of steatorrheic subjects experience increasing diarrhea as their fat intake is raised. If annoying diarrhea is present, fat restriction to 40 gm. or less daily is advisable. To protect the patient with chronic steatorrhea from hypoprothrombinemia, daily oral water-soluble vitamin K derivatives are required (menadione, 5 to 10 mg.); rarely, this compound must be injected to guarantee absorption. Patients with continuing gross steatorrhea require protection also against osteomalacia; calcium balances are usually negative [*137,158*]. Very large intake of *both* calcium and vitamin D is required for a positive calcium balance [*27,158,159*]. Calcium lactate and calcium gluconate contain a very small proportion of calcium; supplements of 5 to 15 gm. daily are usually advisable. An adequate oral dosage (25,000 to 600,000 units) of fat-soluble vitamin D is generally effective. The administration of water-miscible vitamin D emulsions, injectable vitamin D preparations or ultraviolet radiation of the skin may be useful although they are usually unnecessary. The amount of vitamin D given must be adapted to the severity of the tetany, osteomalacia or steatorrhea, and constantly checked to avoid over- or underdosage; frequent repetition of the urine Sulkowitch test and determinations of serum calcium, phosphorus and alkaline phosphatase levels are indicated. The evidence for improving fat absorption with oral emulsifying agents (such as Tween 80) [*160*] is conflicting. Dosage advised by advocates of tween 80 is from 5 to 10 gm. daily, administered in divided amounts with meals. Anticholinergic drugs, such as pamine,® banthine® or pro-banthine,® tend to reduce abnormal gastrointestinal motility in some steatorrheic patients and are therefore useful to decrease painful peristaltic spasm; oral calcium carbonate may do likewise. Opiates, because of potential problems of addiction in chronic disease, should be employed with great caution.

If the malabsorptive defect is diffuse, severe, and involves simple water-soluble compounds, then supplementation with additional nutrients is indicated [*137*]. These include selective combinations of oral or parenteral vitamin B complex, folic acid and vitamin B_{12}. Iron-lack anemias generally necessitate replenishment of iron stores by injectable iron preparations, since oral iron may be non-absorbed. A profuse, watery diarrhea usually requires careful attention to fluid and electrolyte balance. Extreme states of sodium and potassum losses have been observed in sprue syndromes [*137*]. Water deficit also results [*186*].

Post-Gastrectomy Syndromes. Malnutrition in most of these conditions is not due primarily to true malabsorptive defects. It is the result of inability to eat normal quantities of food because of (a) feelings of early fullness in the small gastric reservoir, or (b) the "dumping" syndrome [*56,58,59,161–163*]. Therapeutic strategy is therefore directed toward increasing total caloric intake with limited bulk, but its success is unfortunately limited. Providing a menu of concentrated foods containing readily soluble, osmotically active nutrients will exaggerate a dumping syndrome [*164*]. For those patients having, in addition, true malabsorptive defects (all total gastrectomies, 10 to 15 per cent of

partial gastrectomies, some simple pyloroplasties), no specific drugs have been found regularly helpful [*193*]. An example of this important point is presented in Figure 1. Such negative results are similar to those reported for malabsorptive syndromes following extensive resections of the small bowel [*165,166*]. Supplements of large quantities of pancreatic extract or of certain bile salt preparations have been reported to improve postgastrectomy steatorrhea by a few investigators [*53,64,65*], but quite negative results from somewhat lesser amounts of these supplements have been the regular experience of most investigators [*193*]. ACTH or cortisone acetate have not been effective [*167*]. All patients who have had a total gastrectomy should receive parenteral vitamin B_{12} [*194*] regularly, since they cannot absorb this compound through complete absence of intrinsic factor [*48*] and therefore pernicious anemia would develop.

Exocrine Pancreatic Insufficiency. Only in this condition has pancreatin been consistently demonstrated to be of regular value. Commercial preparations are frequently impotent in enzyme activity. Both enteric-coated and plain forms are available; both have been shown to be effective [*69*]. Dosage varies somewhat with the severity of the exocrine deficiency; usually 8 to 25 gm. daily are needed in adults [*31,69,168*], and will approximately halve the fecal fat and nitrogen losses. The variable response to a standard dose by different patients is presented in the balance studies of Figure 2. In the human subject supplements of choline or other lipotropic agents for preventing fatty liver, such as needed in dogs following pancreatectomy [*169–171*], are thought unnecessary [*70*].

Sprue and Celiac Syndromes. Specific oral supplements of folic or folinic acid (10 to 20 mg. daily) are believed often effective in alleviating "tropical" sprue [*99,109,172*]; frequent failures in "non-tropical" sprue are well recognized [*173*]. The old, traditional liver extracts presumably contained folic acid, folinic acid or vitamin B_{12} as diluted active ingredients; therapeutic failures from liver extracts in "non-tropical" sprue are likewise well documented [*137,159*]. A gluten-free diet (especially omitting wheat glutens) has been reported to improve many of the celiac patients and also certain patients with adult sprue [*157,174–180*]. At times, however, these improvements are slow, partial, and no better than may occur spontaneously in these syndromes, and most observers have found frank failure in parallel with seeming striking benefit. In very acute or resistant chronic states the administration of steroid drugs (ACTH, cortisone acetate, hydrocortisone, prednisolone) may yield excellent improvement [*181–186*]; a sudden and dramatic increase in absorption may occur within twenty-four hours of initiating such therapy, so as to be considered "life-saving" [*187*]. The presence of nutritional edema is not a contraindication to such use of steroids in our experience. The mechanism of improvement by steroids is unknown, but virtually all materials traversing the small bowel mucosal surface may become better absorbed. In comparison with other diseased conditions wherein the non-specific anti-inflammatory effects of steroid agents are sought, the primary sprue syndromes are often excellently regulated by relatively small doses of these drugs; a daily oral maintenance dosage of 25 to 50 mg. cortisone acetate or 5 to 10 mg. prednisolone frequently suffices. Relapse commonly occurs within two to three weeks after omitting steroid therapy. We and others have sometimes noted similar improvement from steroid compounds in the malabsorptive states associated with regional enteritis and Whipple's disease [*94–96, 167*].

SUMMARY

The broad syndromes of gastrointestinal malabsorption are discussed, with special reference to etiology, pathologic physiology, differential diagnosis, laboratory investigation and principles of treatment.

Selected important points in therapy are illustrated with detailed balance studies.

A classification of the steatorrheas based upon the biochemical processes involved in normal fat digestion and absorption is presented.

Acknowledgment: The author is indebted to Mrs. Dorothy Shook for excellent technical assistance.

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Seminar on Atherosclerosis

The Lipoproteins of Human Serum*

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THE lipids of the plasma, consisting of cholesterol, cholesterol esters, phospholipids and triglycerides, are either insoluble or only slightly soluble in water and yet are present in high concentration in clear solutions in the plasma, and readily enter into metabolic processes. Early studies [*1–3*] had suggested the existence of some relationship between these lipids and plasma proteins but it was not until 1929 that Macheboeuf [*4*] isolated a well-defined serum lipoprotein. Upon acidification to pH 3.9 of the horse serum globulins not precipitated by half-saturated ammonium sulfate at neutral pH, he obtained a fraction which was of constant lipid and protein composition on repeated reprecipitation. This material was subsequently found to be an α_1-globulin and has a chemical composition similar to that of α_1-lipoproteins from other species. In 1941 Blix, Tiselius and Svensson [*5*] obtained protein fractions by free electrophoresis and analyzed them for cholesterol and phospholipid. They found that the α- and β-globulin fractions contained the bulk of the serum cholesterol and phospholipid, although the other fractions did contain some lipid. The first to take advantage of the fact that lipoproteins are of lower density than other plasma proteins was Pedersen who in 1947 [*6*] isolated a lipoprotein by ultracentrifugal flotation in a medium of density 1.04 gm./ml. This protein was found to be a β-globulin. In the large scale fractionation of plasma proteins carried on in Cohn's laboratory at Harvard during World War II it was observed that the serum lipids, instead of remaining in the ethanol-water mixture from which the proteins had been precipitated, were always found in two readily separable protein precipitates which readily dissolved to give clear solutions of lipoproteins of high lipid content [*7*].

As the result of these studies there is now general recognition of the fact that virtually all the lipids of the serum are present as constituents of lipoproteins. Any investigation of the chemistry and functional role of the serum lipids necessitates consideration of this fact.

TABLE I
METHODS OF MEASURING SERUM LIPOPROTEINS

I.	Differential solubility
	A. Salting out
	B. Cohn fractionation
II.	Electrophoresis
	A. Moving boundary electrophoresis
	B. Zone electrophoresis
	1. Paper electrophoresis
	2. Starch electrophoresis
III.	Ultracentrifugation
	A. Analytic ultracentrifugation
	B. Preparative ultracentrifugation
	1. Of serums
	2. Of lipoprotein-enriched fractions
IV.	Miscellaneous methods
	A. Immunochemical
	B. Turbidimetric

THE DETERMINATION OF SERUM LIPOPROTEIN CONCENTRATIONS

The methods for determining serum lipoprotein concentrations usually require separation of the lipoprotein fractions and then analysis of the several fractions. In Table I are listed the various methods that have been used for determination of lipoproteins. The earliest methods were based upon the differential solubility of these fractions. The salting out methods with ammonium sulfate, used by Macheboeuf [*4*] and by Adair and Adair [*8*], are no longer in general use. Cohn fractionation [*9*] is a system for precipitation of the plasma proteins in cold ethanol and it requires careful control of ethanol concentration, pH, ionic strength, protein concentration and temperature. By this procedure it is

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possible to separate the serum lipoproteins into two fractions, one containing lipoproteins with electrophoretic mobility of α_1-globulins and the other with the mobility of β_1-globulins. The lipoprotein content of each fraction may then be determined by analysis of the fraction for lipid. Numerous studies of serum lipoprotein concentrations, both in normal subjects and in certain disease states have been carried out [*10–14*]. When performed with care, the results are reproducible and have been very informative. The method is time consuming, however, and there are many opportunities for error. Furthermore, it separates only two lipoprotein fractions and the lipoproteins are not completely separated from other plasma proteins. The lipid-rich fractions can be subjected to ultracentrifugation for further purification [*13,15,16*].

Moving boundary electrophoresis has been used to collect lipoprotein fractions [*5*] but it is not possible to collect homogeneous fractions and the method has been discarded. Analytic moving boundary electrophoresis is of little value in mixtures such as whole serum, since it is not possible to differentiate the lipoproteins from the other proteins. Zone electrophoresis on fixed media, such as paper or starch, has been widely used for the determination of serum lipoproteins [*12,17–20*]. Paper electrophoresis need not require elaborate apparatus and it uses only a small sample of serum. The lipids may be stained and the strips scanned directly or they may be eluted and estimated. Direct scanning is not very accurate; the base line varies and there is considerable uncertainty as to the true base line. Furthermore, the relationship between dye concentration and optical density on paper is non-linear. Elution is somewhat more accurate but is, nevertheless, only semi-quantitative, since the dye binding of the lipids varies considerably and it is impossible to convert the results into chemical units [*21*]. For accurate quantitation in paper electrophoresis it is necessary to elute the lipid from the paper and estimate it chemically [*20,22*]. This unfortunately, removes a good deal of the simplicity of the method, since quantitative elution is difficult and the small amounts of lipid obtained require the use of micromethods.

Starch block electrophoresis has the advantage that lipoproteins are not absorbed to the supporting medium [*18*]. Since larger samples are used, conventional methods for lipid analysis can be employed. The use of this method for the determination of lipoprotein concentrations is limited by the fact that quantitative elution from the starch is difficult [*23*] and, further, that the α_1- and β_1-lipoproteins cannot be subfractionated.

Ultracentrifugal flotation is the most specific of the methods of lipoprotein separation. In the methods developed by Gofman and his coworkers at the Donner Laboratory and described in detail by De Lalla and Gofman [*24*], the lipoproteins are first separated from serum by preparative ultracentrifugation and then analyzed by analytic ultracentrifugation. Refractive index photographs are obtained in which the area under the peak is a function of the lipoprotein concentration. Unfortunately, accurate quantitation of these photographs is most complicated. In addition to problems of base line selection and the necessity of making measurements on components that are only partially resolved, there are the problems of the proper choice of the refractive index gradient to use in the calculations. What appears to be an especially difficult problem is that concerned with the effect of concentration of protein on the results [*24*]. The difficulties of the method are well demonstrated in the data presented by the Cooperative Study of Lipoproteins and Atherosclerosis [*25*]. After over two years of work on the project, S_f 12–20 values on the same sample varied from 23.2 mg. per 100 ml. at Harvard to 34.6 at the Donner Laboratory. The S_f 12–100 results on the same sample varied from 38.8 at Harvard to 60.0 at Donner. Serum cholesterol determinations varied from 143 mg. per cent to 167 mg. per cent. It seems reasonable to conclude that this method will not have general usefulness for the determination of the concentrations of serum lipoproteins.

In 1951 Lindgren, Elliott and Gofman [*26*] described the separation of serum lipoproteins with the preparative ultracentrifuge by successively raising the solvent density. These fractions could then be analyzed chemically. A similar procedure has been described by Hillyard et al. [*27*]. Havel, Eder and Bragdon [*28*] described a method similar to that of Lindgren with ultracentrifugal flotation carried out at $D < 1.019$, D 1.019–1.063, and D 1.063–1.21. This latter fraction was on occasion subfractionated. With this technic it has been possible to recover 98 per cent of the total serum cholesterol and 99 per cent of the total serum phospholipids in the isolated fractions. The serum concentrations of these components can be

estimated from the lipid analyses. This technic is relatively simple and affords an excellent measure of serum lipoprotein concentrations.

An ingenious modification of this technic is that employed by Oncley and Mannick [29] in which a number of lipoproteins are separated by a single centrifugation in a gradient density tube. It is possible by this technic to isolate several groups of lipoproteins simultaneously, whereas with other preparative procedures a separate run in the centrifuge is necessary for each fraction isolated.

As previously mentioned, Cohn fraction I + II + III has been subjected to preparative ultracentrifugation for further isolation of lipoproteins. Recently, Oncley [30] has described a method for isolation of lipoproteins based on the fact that a high molecular weight dextran sulfate will precipitate β-lipoprotein from serum. The lipoprotein is then readily separated from the dextran sulfate and subjected to ultracentrifugation in a gradient density tube.

Other methods have also been proposed for the determination of lipoprotein concentrations. Since antibodies against the lipoproteins can be produced, it has been suggested that lipoproteins be determined by immunochemical methods [31]. Bernfield has suggested that β-lipoprotein be determined by the nephelometric measurement of the turbidity formed when β-lipoprotein reacts with amylopectin [32].

NOMENCLATURE OF LIPOPROTEIN FRACTIONS

In this discussion, data on lipoprotein fractions obtained by a variety of analytic methods will be presented. In general, these fractions will be identified according to the recommendations made by the Committee on Lipid and Lipoprotein Nomenclature of the American Society for the Study of Atherosclerosis [33] that each fraction be described in the terms of its method of separation, e.g., S_f is to be confined to flotation rates at density 1.063; α- and β-lipoproteins are to be confined to electrophoretic analyses; and lipoproteins separated by Cohn fractionation are to be designated by the appropriate fraction number. Fortunately, it is possible to relate these different methods and this has been done in Table II. The low density lipoproteins (D < 1.063) correspond to the Gofman S_f classes >0. All these lipoproteins have the electrophoretic mobility of β-globulins except the D < 1.006 chylomicrons, which have the mobility of α_2-globulins on starch [34]. The lipids in Cohn fractions I + III correspond closely to those of D < 1.063. The lipoproteins of D > 1.063, the high density lipoproteins, have the mobility of α_1-globulins and are usually found in Cohn fraction IV + V.

TABLE II
INTERRELATIONSHIP OF VARIOUS METHODS OF LIPOPROTEIN DETERMINATION

Density	S_f [24]	−S [59]	Starch Electrophoresis [18]	Cohn Fraction [9]
Low Density Lipoproteins				
<1.006 (chylomicrons)	>20	>70	α_2	I+III
1.006–1.019	12–20	40–70	β_1	I+III
1.019–1.063	0–12	25–40	β_1	I+III
High Density Lipoproteins				
1.063–1.125	HDL_2	20–25	α_1	IV+V
1.125–1.21	HDL_3	1–10	α_1	IV+V

CHEMICAL AND PHYSICAL PROPERTIES OF THE LIPOPROTEINS

Lipid Composition. Analyses of the serum lipoprotein fractions have been made by Bragdon et al. [35] and these data are shown in Table III. The fractions of D < 1.063 differ considerably from those of D > 1.063, in that the latter have a considerably higher protein content, and also they have cholesterol-phospholipid ratios of *ca.* 0.5, as compared to ratios of 1.0 or over in the low density group. In the low density fractions (D < 1.063) there is a progressive increase in triglyceride content as the density decreases. In these low density fractions the ratios of free to total cholesterol and of cholesterol to phospholipid vary consistently from one fraction to another.

The high density fraction (D > 1.063) can be divided into two fractions, one of density 1.08 and the other of density 1.15 [24]. This higher density fraction has a higher protein content [28].

Somewhat similar studies have been carried out by Lindgren et al. [36] who separated lipoprotein fractions, then separated the lipids by silicic acid chromatography, and finally analyzed the fractions by infra-red absorption. Their results differ only slightly from those of Bragdon et al.

TABLE III
THE PER CENT COMPOSITION OF SERUM LIPOPROTEIN FRACTIONS [35]

Fraction	Free Cholesterol	Cholesterol Esters	Phospholipid	Triglyceride	Protein	FC/TC*	TC/PL†
D < 1.006 (chylomicrons)	3.1	6.0	7.1	81.3	2.5	0.46	0.95
D < 1.019	6.0	16.2	17.9	51.8	7.1	0.37	0.90
1.019–1.063	7.5	39.4	23.1	9.3	20.7	0.24	1.30
1.063–1.21	2.0	17.4	26.1	8.1	46.4	0.16	0.48

* FC/TC = Free cholesterol/total cholesterol.
† TC/PL = Total cholesterol/phospholipid.

Some attempts to characterize the lipids in the lipoprotein fractions have been made. Steele and Kayden [37] studied the phospholipid composition of α- and β-lipoproteins and found that there was a higher proportion of sphingomyelin to lecithin in the β fraction than in the α fraction. Gillies et al. [38] have determined the fatty acid composition of the lipoproteins of the S_f 0–20 and 20–400 classes. They found that the S_f 0–20 fraction contained large amounts of linoleic acid in addition to stearic, palmitic and oleic acids. The S_f 20–400 fraction contained stearic, palmitic, oleic and palmitoleic acids but no linoleic acid. These differences are probably due to the fact that cholesterol, which is esterified chiefly with linoleic acid [39], is present in largest amounts in the S_f 0–20 fraction whereas the fatty acids in the S_f 20–400 group derive primarily from triglyceride. A study of fatty acid composition would be more meaningful if the fatty acid composition of the various lipid complexes which contain fatty acids were compared, rather than the total hydrolysate of these lipids.

Protein Composition. Recently Avigan, Redfield and Steinberg [40] determined the amino acid composition of the N-terminal residues of lipoproteins by preparing dinitrophenyl derivatives. They showed that in the D 1.019–1.063 fraction, glutamic acid appears to be N-terminal, whereas in the D 1.063–1.21 fraction, aspartic acid is N-terminal. Studies by Shore [41] have also shown glutamic acid to be N-terminal in the D 1.029 lipoprotein and aspartic acid to be N-terminal in the D 1.093 and 1.149 lipoproteins. In the D > 1.006 fraction serine, glutamic acid and threonine were present in the N-terminal position. Threonine was found to be the principal C-terminal amino acid of the high density lipoproteins, while serine is probably C-terminal in the D 1.029 fraction. In the D > 1.006 lipoproteins, alanine was C-terminal in at least one peptide chain and serine may possibly be C-terminal in another.

Immunochemical Studies. Levine et al. [42] have shown by quantitative precipitin reactions, complement fixation and diffusion in agar that the low density lipoproteins are antigenically similar and do not react with high density α-lipoproteins. Middleton [43] showed that the protein in the chylomicrons would react with antibody to S_f 3–8 lipoprotein if the chylomicrons were pretreated with lipase. Aladjem et al. [44] have also shown that the S_f 6 and S_f 13 lipoproteins are antigenically similar and that they differ from the high density lipoproteins, although one of the high density fractions did have a component also found in the low density lipoproteins.

Physical Characteristics. The high density lipoproteins have molecular weights ranging from 165,000 to 435,000 [41]. The low density lipoproteins have much higher molecular weights, the lowest being about 2,100,000 (hydrated) [16] and this may increase to as high as 250 million for the chylomicrons [41]. The physical characteristics of the lipoproteins separated in the Cohn fractions have been described by Oncley, Scatchard and Brown [45]. They showed that β-lipoprotein is 60 per cent water by weight and that this water has an important structural role in the molecule since, if removed, much of the lipid splits off. From their measurements they concluded that the molecule was a sphere with the diameter of 185 Å. They suggested that since the molecule has so many of the properties of a protein, the polypeptide chains must be arranged on the surface with the lipid inside. They concluded that the α-lipoprotein is ellipsoid in shape, with a long axis of 300 Å and a short axis of 50 Å.

From these chemical and physical data it is

apparent that the low density lipoproteins constitute a group of closely related proteins with many similar properties. The protein portion of this group is certainly very similar and the subfractions differ from one another chiefly in their lipid content. The high density lipoproteins differ markedly from the low density lipoproteins, both in lipid composition and in the protein portion of the molecule.

METABOLISM OF THE SERUM LIPOPROTEINS

The earliest workers in the study of lipoproteins suggested that because of their high fat content the lipoproteins must play an important role in fat transport [*5,6*]. However, definitive information is available only concerning the metabolic functions of the low density chylomicrons. After the oral administration of fat to human subjects, Jones et al. [*46*] have demonstrated a rise in the low density ($S_f > 60$) fraction, followed by a stepwise conversion to the adjacent higher density fractions. Havel and Fredrickson [*47*] approached this problem directly by feeding dogs palmitic acid-1-C^{14} and obtaining chyle from the thoracic duct. This chyle was injected intravenously into other dogs and it was found that the chylomicra disappeared rapidly, with a half-life ranging from eleven to twenty-four minutes. Recently, we carried out a similar experiment in which "chyle" with the phospholipids labeled with P^{32} was obtained by administering P^{32} to a patient with chylous ascites. This ascitic fluid was administered to a patient with a carcinoma of the pancreas and the disappearance of lipids was determined both by chemical and isotopic measurements. The serum triglyceride concentrations at first fell rapidly, with half maximal activity reached in fifteen minutes, but there was a second and slower component in which half maximal activity was not reached until two hours. P^{32} activity decreased at about the same rate. These data suggest that chylomicra are rapidly removed from the circulation. Under normal conditions the chylomicron fraction consists largely of ingested lipid and the function of this fraction is primarily one of transport of this lipid to storage depots.

The functions of the remaining fractions of lipoproteins have yet to be ascertained. In the process of investigating this problem a good deal has been learned about the nature of the lipoproteins. One useful means of investigating the function of the various lipoproteins would appear to be comparison of the rates of incorporation and turnover of various precursors in the lipoproteins. We [*48,49*] and others [*50*] attempted this with P^{32}, both in human subjects and in rabbits, and found that the activity of the P^{32} in the various fractions was virtually identical at every period. In Table IV are shown the results of *in vitro* mixing experiments. It is apparent that there is appreciable exchange of phospholipid phosphorus between the major groups of lipoproteins. Whereas the phospholipid exchanges readily *in vitro* between the clear lipoprotein fractions, we found that when labeled chylomicrons were mixed with plasma virtually no exchange of P^{32} activity occurred. That this exchange involves more of the molecule than the phosphorus has been shown by incubating the serum with inorganic $P^{32}O_4$ and finding no activity in the lipoprotein fractions. Gidez and Eder, using glycerol-C^{14} labeled phospholipids [*51*], have found *in vitro* exchange between lipoprotein fractions and this suggests that the entire phospholipid molecule does exchange. The fact that there is exchange between the plasma free cholesterol and red cell cholesterol [*52*] makes cholesterol exchange between lipoproteins likely. Chyle triglyceride did not exchange *in vitro* with plasma lipoproteins [*47*].

TABLE IV
IN VITRO MIXING OF LABELED LIPOPROTEINS WITH UNLABELED LIPOPROTEINS

Labeled Fraction	Incubation Time (hr.)	Counts per Minute/μgP after Mixing	
		D < 1.063	D > 1.063
D < 1.063	0	22.0	3.9
D < 1.063	3½	18.0	8.1
D < 1.063	21	14.1	9.7
D < 1.063	21 at 4°c.	18.0	7.5
D > 1.063	0	2.3	20.7
D > 1.063	3½	3.5	12.4
D > 1.063	21	5.4	9.2
D > 1.063	21 at 4°c.	3.1	15.2

The synthesis of the lipid portion of the lipoproteins has also been studied by measuring the incorporation of glycerol-1,3-C^{14} into the triglycerides and phosphatides of a patient with idiopathic hyperlipemia [*51*]. The triglyceride of the D 1.006–1.063 fraction reached a specific activity considerably higher than that in the

chylomicrons or in the high density lipoproteins and it turned over at a more rapid rate. Furthermore, the triglyceride glycerol differed in activity in each of the three lipoprotein fractions and this suggested that the triglyceride does not exchange freely between the lipoproteins. With phospho-

TABLE V

MEAN VALUES FOR HIGH DENSITY LIPOPROTEINS (MG. PER 100 ML.) IN NORMAL HUMAN SUBJECTS

Age (yr.)	Analytic Ultracentrifuge*	Preparative Ultracentrifuge†	Cohn Fractionation‡	Paper Electrophoresis§
		Males		
20–39	255	346	419	427
40–65	259	...	427	419
		Females		
20–39	321	435	480	508
40–65	323	...	427	468

* From DE LALLA, O. F., ELLIOTT, H. A. and GOFMAN, J. W. *Am. J. Physiol.*, 179: 333, 1954 [*58*] (sum of D 1.075 + D 1.145 lipoproteins).

† From HAVEL, R. J., EDER, H. A. and BRAGDON, J. H. *J. Clin. Investigation*, 34: 1345, 1955 [*28*], calculated from cholesterol.

‡ From RUSS, E. M., EDER, H. A. and BARR, D. P. *Am. J. Med.*, 11: 468, 1951 [*10*], calculated from cholesterol.

§ From NIKKILÄ, E. *Scandinav. J. Clin. Lab. Invest.* (Suppl. 8), vol. 5, 1953 [*19*], calculated from cholesterol.

lipid glycerol the highest specific activity was found in the chylomicrons and it turned over in that fraction more rapidly than in the other two fractions. In the D 1.063–1.21 and in the D 1.006–1.063 fractions the phospholipid glycerol turned over very slowly. In those two fractions the specific activities were virtually identical, suggesting that phospholipids do exchange. These studies show that in idiopathic hyperlipemia there is some endogenous synthesis of chylomicron triglyceride and phospholipid as well as synthesis of the lipids in the other lipoproteins. These studies of *in vitro* exchange of lipid between the lipoproteins indicate that certainly in the case of phospholipid the chemical bond between the lipid and the protein is relatively loose. Phospholipid can enter and leave the molecule freely so its metabolic activities are, therefore, to some extent independent of the remainder of the lipoprotein molecule. That the triglyceride is not so freely exchangeable is not unexpected, in view of its low solubility in aqueous solutions as compared to phospholipid.

In the study of the metabolism of the lipoproteins, it is at least as important to study the protein moiety as the lipid moiety. Volwiler et al. [*53*] labeled β-lipoproteins in man, both by feeding S^{35}-cystine and by injecting biosynthetically S^{35}-labeled human plasma. With the former procedure the half-life for β-lipoprotein was about six days and in the latter, 3.2 days. Avigan, Eder and Steinberg [*49,54*] measured the incorporation of alanine-1-C^{14} into the lipoproteins of rabbits and found that the D < 1.063 and D 1.063–1.21 fractions of the lipoproteins had a maximal half-life of 2.5 days. The D < 1.063 fraction had an early phase of rapid decrease in activity, the time of half maximal activity being reached in nine hours. They could find no evidence for interconversion of the lipoproteins of the D < 1.063 and D 1.063–1.21 fractions. Gitlin et al. [*55*] labeled human serum lipoproteins with I^{131} and found that the S_f 3–8 group had a half-life of slightly over three days, the S_f 15–400 had a more rapid turnover rate, and the α-lipoproteins had a half-life of between four and five days. Of great importance is their observation [*56*] that a large part of the disappearance of the S_f 15–400 lipoproteins is accounted for by conversion into the higher density S_f 3–8 class. In patients with the nephrotic syndrome this conversion was markedly diminished.

Thus there is evidence from several investigations that the protein moieties of the lipoproteins turn over more rapidly than any of the other serum proteins. The low density β-lipoproteins probably turn over more rapidly than do the high density α-lipoproteins. It seems likely that these relatively rapid rates of turnover are related to the transport role of the lipoproteins. The importance of this transport role of the lipoproteins requires more investigation. The data of Gordon and Cherkes [*57*], indicating that unesterified fatty acids bound to albumin are the blood lipid fraction primarily concerned with the supply of fat to the tissues for oxidative metabolism, adds more uncertainty as to the role of the lipoproteins.

NORMAL SERUM LIPOPROTEIN CONCENTRATIONS

In Table V are shown the serum concentrations of the high density lipoproteins in males and females in the age groups twenty to thirty-

nine and forty to sixty-five. The data from the Donner Laboratory were obtained by analytic ultracentrifugation and are the sums of the concentrations of the lipoproteins of D 1.145 and D 1.075. The cholesterol values obtained by preparative ultracentrifugation [*28*], Cohn fractionation [*10*] and paper electrophoresis [*19*] have been converted to lipoprotein concentrations by dividing the cholesterol by 0.124, the cholesterol content of the isolated high density lipoproteins. While the lipoprotein concentrations estimated from cholesterol determinations are quite similar, the values by analytic ultracentrifugation are slightly lower. The females all have higher concentrations of these lipoproteins than do the males. No changes with age are seen in the males but the data from Cohn fractionation and from electrophoresis show a decreased concentration of these lipoproteins in the female with aging. Values obtained by ultracentrifugal flotation at D 1.21 by Lewis and Page [*59*] are considerably lower than those tabulated.

In Table VI are shown values for the low density lipoproteins in normal subjects obtained by ultracentrifugation, analytic [*60*] and preparative [*28*]. The cholesterol values have been converted to lipoprotein concentrations using the data in Table III. Data from Cohn fractionation and electrophoresis cannot be compared because only a single fraction is isolated in both these procedures. The data from the Donner Laboratory show that young males have higher low density lipoprotein concentrations than do young females, and the older males and females have higher concentrations than young males and females. These findings are similar to those found by Barr, Russ and Eder [*14*] using Cohn fractionation and those of Nikkilä [*19*] using electrophoresis.

From these data it may be seen that the serum lipoproteins constitute slightly more than 10 per cent of the total serum proteins. Between 30 and 40 per cent of the total serum lipoproteins is normally present as high density lipoproteins. About 40 per cent is present as the D 1.019–1.063 fraction and the remainder is present in the very low density fraction.

SERUM LIPOPROTEIN CONCENTRATIONS IN ATHEROSCLEROSIS AND RELATED DISEASES

Many workers have shown that the concentrations of serum cholesterol are increased in groups of patients with atherosclerosis but that many individual patients have serum cholesterol values well within the normal range. When in 1950 Gofman et al. [*61*] described alterations in serum lipoprotein concentrations in patients with atherosclerosis, investigation of this question was undertaken by a variety of methods. A small fraction of the data obtained in these investigations is summarized in Table VII. The data obtained by analytic ultracentrifugation from the Donner Laboratory [*60*] concern the S_f 0–12 and S_f 12–400 classes, which cover almost the entire range of low density lipoproteins, while the data of Lawry et al. [*62*] are limited to the S_f 12–20 and S_f 20–100, which were earlier thought by the Donner group to be the most significant. The data from Cohn fractionation and paper electrophoresis cover the entire range of the low density, beta lipoproteins. In all these data the mean lipoprotein or cholesterol concentrations are higher in the patients with atherosclerosis than in the normal population. Where adequate data over a broad age range are given [*60,62*] the differences are most marked in the younger age groups. The question of the significance of such observations in relation to

TABLE VI
MEAN VALUES FOR LOW DENSITY LIPOPROTEINS (MG. PER 100 ML.) IN NORMAL HUMAN SUBJECTS

Age (yr.)	D 1.019–1.063		D 1.019	
	Analytic Ultracentrifuge*†	Preparative Ultracentrifuge‡	Analytic Ultracentrifuge*§	Preparative Ultracentrifuge‡
	Males			
20–39	357	312	221	166
40–65	381	...	235	...
	Females			
20–39	298	347	136	123
40–65	357	...	203	...

* From GOFMAN, J. W., GLAZIER, F., TAMPLIN, A., STRISOWER, B. and DE LALLA, O. *Physiol. Rev.* 34: 589, 1954 [*60*].

† S_f 0–12.

‡ From HAVEL, R. J., EDER, H. A. and BRAGDON, J. H. *J. Clin. Investigation*, 34: 1345, 1955 [*28*], calculated from cholesterol.

§ S_f 12–400.

TABLE VII

MEAN VALUES FOR LOW DENSITY LIPOPROTEINS (MG. PER 100 ML.) IN MALES WITH ATHEROSCLEROSIS (A) AND IN NORMAL MALES (N)

Age (yr.)	S_f0–12*		S_f12–400*		S_f12–20†		S_f12–100†		Cholesterol in Fraction I + III‡		β_1-Lipoprotein Cholesterol§	
	N	A	N	A	N	A	N	A	N	A	N	A
30–39	...	...	...	...	36	59	81	117	142	233	...	...
40–49	385	420	223	320	39	44	87	97	...	229	...	...
50–59	408	431	234	317	39	51	82	114	180	...	228	273
60–69	396	404	217	239	37	39	76	86	...	...	...	...

* From GOFMAN, J. W., GLAZIER, F., TAMPLIN, A., STRISOWER, B. and DE LALLA, O. *Physiol. Rev.*, 34: 589, 1954 [*60*].

† From LAWRY, E. Y., MANN, G. V., PETERSON, A., WYSOCKI, A. P., O'CONNELL, R. and STARE, F. J. *Am. J. Med.*, 22: 605, 1957 [*62*].

‡ From BARR, D. P., RUSS, E. M. and EDER, H. A. *Am. J. Med.*, 11: 480, 1951 [*14*].

§ From JENCKS, W. P., HYATT, M. R., JETTON, M. R., MATTINGLY, J. W. and DURRUM, E. L. *J. Clin. Investigation*, 35: 980, 1956 [*63*].

the diagnosis and prediction of atherosclerosis in patients will be discussed in detail. It should be emphasized, however, that irrespective of any such application the differences shown in Table VII undoubtedly have considerable significance as to the pathogenesis of atherosclerosis

TABLE VIII

LIPOPROTEINS IN THE NEPHROTIC SYNDROME (CHOLESTEROL, MG. PER 100 ML.)

Subjects	Serum	D < 1.019 S_f > 9	D 1.019–1.063 S_f 3–9	D > 1.063 α
Normal Subjects				
	189	30	106	43
*Nephrotic Subjects**				
I	477	255	137	78
II	1,058	907	90	82
III	520	354	66	25
IV	547	408	92	26
V	980	774	64	15
VI	570	419	60	30
VII	884	624	141	22

* Nephrotic subjects I and II are from HAVEL, R. J., EDER, H. A. and BRAGDON, J. H. *J. Clin. Investigation*, 13: 1345, 1955 [*28*]; III–VIII are from GITLIN, D. Personal communication [*65*].

An associated finding in these patients with atherosclerosis has been an absolute reduction in the concentration of cholesterol in Cohn fraction IV + V [*14*] and in beta lipoprotein determined by paper electrophoresis [*19,63*]. Doyle et al. [*64*] have determined the beta lipoproteins both by Cohn fractionation and by analytic ultracentrifugation at D 1.21 and have been unable to confirm this. It is a point which should be resolved.

A better understanding of the nature of the alterations in serum lipoprotein patterns found in atherosclerosis may be obtained from consideration of lipoprotein distributions in certain of the diseases in which there is a marked predisposition to atherosclerosis. In patients with the nephrotic syndrome Cohn fractionation [*14*] showed that the high serum cholesterol was due to an increase of cholesterol in fraction I + III. When serums from patients with the nephrotic syndrome were separated by preparative ultracentrifugation and the cholesterol in the low density fraction subfractionated (Table VIII) the major increase in cholesterol was in the D < 1.019 fraction. D 1.019–1.063 usually was not elevated and was more often below the normal mean. When these results are calculated in terms of lipoproteins concentrations the mean of the D < 1.019 fraction is over 3500 mg. per 100 ml. With such large increases in the concentration of very low density components rich in triglycerides, serum triglyceride concentrations are markedly elevated and the serum is lactescent.

In patients with familial hypercholesterolemia and with idiopathic hyperlipemia Cohn fractionation reveals an increase in cholesterol in fraction I+III [*14,66*]. When serums from such patients were studied in the analytic ultracentrifuge [*67*] it was found that the patients with tendon sheath xanthomata (presumably familial hypercholesterolemia) had increases in the concentrations of the lipoproteins ($S_f < 20$) and that the lower density lipoproteins were present in normal or even reduced concentrations. In the patients with idiopathic hyperlipemia the S_f 0–20 lipoproteins were present in reduced concentration while the $S_f > 20$ lipoproteins were markedly increased. Cases in which both the higher and the lower density β-lipoproteins were present in increased concentrations also were described. In the patient with xanthoma tendinosum, Havel et al. [*28*] studied by preparative ultracentrifugation, there was a slight increase in cholesterol in the D < 1.019 fraction but the large increase was in the D 1.019–1.063 fraction. In their patient with idiopathic hyperlipemia the great increase in cholesterol was in the D < 1.019 fraction; whereas the cholesterol in the D 1.019–1.063 fraction actually decreased. The pattern found in familial hypercholesterolemia is an exaggeration of that generally seen in patients with atherosclerosis but in certain patients with atherosclerosis the very low density fraction may be appreciably increased. It should be apparent from these observations that if only a small portion of the low density lipoprotein spectrum is examined, abnormalities which occur in other lipoproteins may not be apparent.

In the diseases discussed thus far the lipoproteins found in the serum are similar to those found in normal subjects except that certain of them occur in elevated concentrations. In patients with obstructive jaundice, Eder et al. [*68*] found lipoproteins which differ markedly in their chemical and physical characteristics from the normally occurring lipoproteins. Russ, Raymunt and Barr [*69*] have identified three such lipoproteins, which appear in Cohn fractions I+III, IV+V and VI but have the mobility of β globulins and are of low density. All of them have low cholesterol-phospholipid ratios; a large proportion of their cholesterol is unesterified; and they have proteins contents appreciably lower than those ordinarily found in the low density lipoproteins. Immunochemically they do not react with antibodies to the low density lipoproteins [*42*]. They disappear from the blood rapidly with subsidence of biliary tract obstruction.

CLINICAL SIGNIFICANCE OF SERUM LIPOPROTEIN MEASUREMENTS IN ATHEROSCLEROSIS

It is almost impossible clinically to recognize the presence of atherosclerosis unless the patient suffers some accident, such as a myocardial infarction, which then reveals the presence of atherosclerosis. Any procedure which could reveal the presence of atherosclerosis before such an accident would, obviously, have immense clinical value. Following the statement of Gofman et al. [*61*] in 1950 that in 104 patients with myocardial infarction there was an almost *universal* occurrence of high concentration of the S_f 10–20 class of lipoproteins, the National Advisory Heart Council organized a large scale study of this problem. In addition to the Donner Laboratory, laboratories at the University of Pittsburgh, the Cleveland Clinic, and Harvard undertook the determination of serum lipoproteins by analytic ultracentrifugation using the Gofman procedures. These four laboratories measured the concentrations of the S_f 12–20 and S_f 20–100 lipoproteins and serum cholesterol in a large number of subjects from all over the country. They studied 4,914 "normal" men, aged forty to fifty-nine, in whom a clinical follow-up was obtained at the end of either one or two years. Within this period clinical manifestations of coronary heart disease developed in eighty-two men in this group. The measurements in this group were then compared with those of all 4,914 men.

Three questions were asked of these data: (1) Was there association between the appearance of clinical manifestations of coronary heart disease and prior elevation of total cholesterol and the S_f 10–20 and S_f 20–100 lipoproteins? (2) Did the measurement of lipoproteins have any advantage over the measurement of serum cholesterol in revealing association? (3) Can these determinations predict those individuals in whom coronary heart disease will develop?

The data demonstrated an association between the appearance of clinical manifestations of atherosclerosis and prior elevation of total cholesterol and S_f 20–100 lipoproteins, but there was no significant prior elevation of the S_f 12–20 class. These data are significant in showing that such changes in lipids antedate the presence of coronary heart disease and thus are not a consequence of it. The data differ from those of

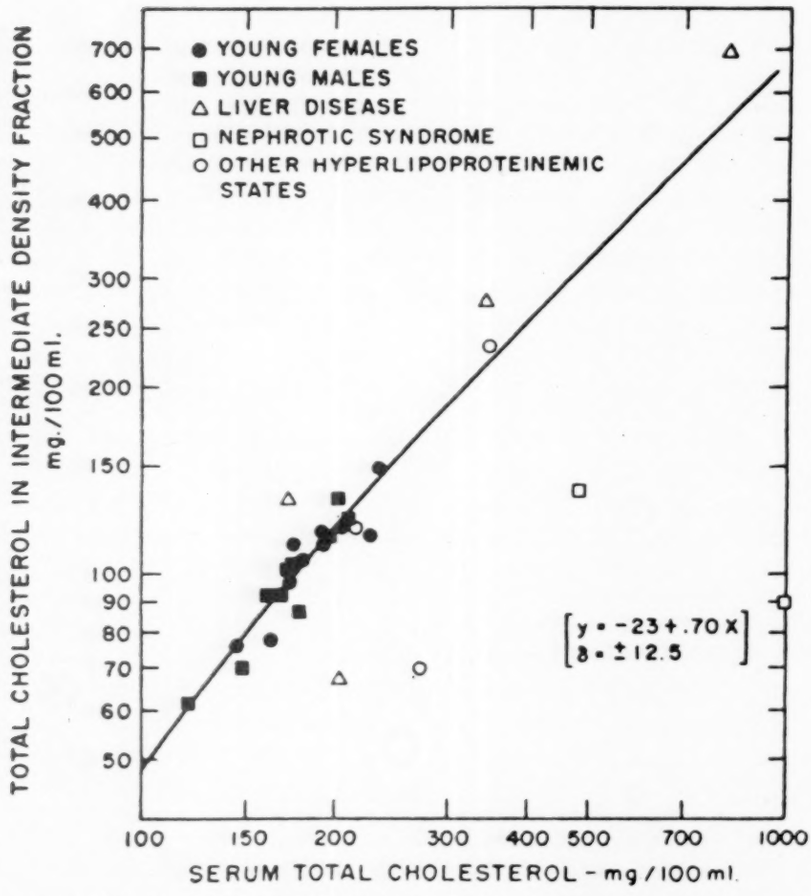

FIG. 1. Relation of total cholesterol in D 1.019–1.063 fraction to total serum cholesterol. Regression line was calculated from data on ten young females and ten young males by the method of least squares. δ = standard deviation of regression. (HAVEL, R. J., EDER, H. A. and BRAGDON, J. H. *J. Clin. Investigation*, 34: 1345, 1955 [*28*].)

Lawry et al. [*62*] shown in Table VII in that the younger age group in which the greatest differences in lipid concentrations between normal subjects and patients with atherosclerosis occur, were not included in the study. The Donner group was dissatisfied with these data: They maintained that the methods of lipoprotein determination, although proposed by them, were obsolete. They believed that a modified procedure [*24*], in which correction for concentration effects are made, should have been used. With data obtained by this procedure they found differences in both lipoprotein classes between the subjects with coronary heart disease and those with atherosclerosis. Although their original study [*61*] had focussed attention upon the S_f 10–20 class of lipoproteins, over the years they had broadened the range of analysis and they now proposed that virtually the entire range of low density lipoproteins, the S_f 0–12 and S_f 12–400 values, be measured. In this group, as in the group shown in Table VII, they conclude that there are significant differences between the normal and the diseased population. Certainly from this study it may be concluded that differences in serum lipoprotein concentrations between groups with atherosclerosis and normal subjects do exist. Furthermore, it is evident that alterations in low density lipoproteins may occur in any or all the subfractions, and that it is therefore not sufficient to pick out any narrow band for study. The reasons for this have been discussed previously. If the total low density fraction is of significance, it would seem that analytic ultracentrifugation would have little advantage over other methods for the measurement of the low density lipoproteins.

From the primary data of this study it was

concluded that the measurements of lipoproteins had no advantage over the measurement of serum cholesterol. As has been indicated in earlier discussion, all the serum cholesterol is present as a constituent of the serum lipoproteins. In atherosclerosis the cholesterol in the α-lipoproteins may decrease, consequently the increase of serum cholesterol is proportionately higher in the low density lipoproteins than in the unfractionated serum. Havel et al. [*28*] (Fig. 1) have shown that there is good correlation between total serum cholesterol and the cholesterol in the D 1.019–1.063 fraction. The exceptions to this were those cases in which there were high concentrations of the D < 1.019 fraction and cases of liver disease in which abnormal lipoproteins were present. This correlation could be anticipated because of the high cholesterol of the D 1.019–1.063 fraction. Similarly, Nichols et al. [*70*] showed that the correlation coefficient between the S_f 0–12 lipoprotein concentrations and total serum cholesterol was 0.87. With the S_f 12–400 fraction the correlation was poorer. When, as in the Cooperative Study, the range of serum lipoproteins studied is limited to the S_f 12–100 classes, which are low in cholesterol, the correlation between lipoprotein concentration and serum cholesterol may be very low. This would be especially true in those patients with atherosclerosis in whom the concentration of the S_f 0–12 lipoproteins, rich in cholesterol, was elevated. In this situation the cholesterol determinations would be much more meaningful than the lipoprotein. On *a priori* grounds it would seem that the measurement of the total low density lipoprotein concentrations should reveal greater association with the presence of atherosclerosis than measurement of total serum cholesterol. The data of Barr, Russ and Eder [*14*] suggest that this is true.

An important clinical corollary of this discussion is the fact that it is possible to make a fairly good prediction of serum lipoprotein patterns from total serum lipid measurements. In patients with cholesterol-phospholipid ratios of *ca.* 0.5 and high free-to-total cholesterol ratios, the presence of the abnormal lipoproteins associated with obstructive jaundice may be suspected. In other patients with high concentrations of cholesterol and phospholipid and slight elevation of serum triglyceride concentration, increased concentrations of the D 1.019–1.063 lipoproteins should be suspected. On the other hand, with high triglyceride concentrations with lactescent serum and moderate elevations of the cholesterol and phospholipid, increases in the concentration of the D < 1.019 lipoproteins would be expected.

The third question investigated was the predictive value of serum lipid determinations. This was the area of most disagreement between the Donner group and the three Eastern laboratories. The basis of the disagreement is probably a difference in the interpretation of the meaning of prediction. The Eastern laboratories have considered prediction in terms of the individual patients, whereas the Donner group have considered it in an actuarial sense. The illustration given in the report [*25*] shows this point clearly. If one considers a hypothetic population of 1,000 well men at ages forty to fifty-nine, there would be expected an incidence, at most, of twenty new occurrences of coronary heart disease during a two-year period of observation. On the basis of this study, between ten and fourteen of the twenty cases would have lipid values above the median for the population and between six and ten would be below the median. These would be the false negatives. Almost 500 men above the median would be the false positives. The fallibility of such a screening measure is apparent. Although by better methodology the extent of overlap between the well and diseased population could, perhaps, be decreased, it still would be great. The problem undoubtedly lies in the selection of the "normal" population. Atherosclerosis not manifested by the clinical presence of coronary artery disease is prevalent in an American population of this age. For recognition of atherosclerosis it may be more reasonable to use, as normal subjects, children, young women and cultural groups with a low incidence of coronary artery disease, as suggested by Lawry et al. [*62*].

SUMMARY

Almost all the serum lipids are present in the plasma as constituents of lipoproteins. On the basis of their chemical and physical characteristics the serum lipoproteins may be separated into two major classes, the low density or β-lipoproteins and the high density α_1-lipoproteins. The low density lipoproteins contain over 80 per cent lipid and have molecular weights over 2,000,000. The high density lipoproteins are only about 50 per cent lipid and have molecular weights of about 200,000. The phospholipid and probably the cholesterol in the lipoproteins exchange freely from one group to another. The

protein portion of the lipoproteins exhibits more rapid rates of turnover than do the other plasma proteins. The low density fraction probably turns over faster than does the high density fraction.

In atherosclerosis and related diseases the low density lipoproteins are increased in concentration. The increases may occur in any or all of the subfractions of the low density group. Analytic methods which measure the entire range of the low density lipoproteins are more useful in demonstrating abnormalities in lipoprotein concentrations in patients with atherosclerosis and related diseases than are methods in which a narrow range is measured. Because of the marked overlap with the "normal," these measurements have not been found to be useful in predicting the appearance of complications of atherosclerosis, such as myocardial infarction, in individual patients.

Study of the serum lipoproteins has been of fundamental importance to our understanding of lipid transport by the blood. Certainly one can no longer consider the various serum lipids as homogeneous entities but, rather, separately, each in terms of the lipoprotein in which it occurs. Under these terms it is possible to consider physiologic and pathologic alterations in serum lipid concentrations more exactly. The association of various diseases with these alterations in lipoproteins may be of the utmost importance in determining their pathogenesis and treatment.

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Combined Staff Clinic

Multiple Myeloma

Current Clinical and Chemical Concepts

THESE are stenotyped reports of Combined Staff Clinics of the College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York. The clinics, designed to integrate basic mechanisms of disease with problems of diagnosis and treatment, are conducted under the auspices of the Department of Medicine. The reports are edited by Dr. Richard J. Cross.

DR. ELLIOTT F. OSSERMAN: This morning, we plan to review and reappraise the clinical features of multiple myeloma, and to consider some of the more recent physicochemical and immunochemical studies of the associated abnormalities in protein metabolism. Let us begin by acknowledging that our orientation will be principally directed toward the characteristic protein abnormalities, as opposed to a straightforward clinical approach. We believe the "characteristic protein abnormalities," which we shall shortly define, are unique and distinctive to an aberration of growth and metabolism of a particular family of cells in which the plasma cell occupies a dominant position. We consider that the demonstration of a "characteristic abnormality" of the proteins in the serum or urine of a particular patient justifies the inclusion of such a case within the disease spectrum of multiple myeloma.

In the past two years we have had the opportunity to study the clinical, pathologic and biochemical features of 100 cases considered to fall within this disease spectrum of multiple myeloma. In ninety-seven cases "myeloma-type" proteins were found in either serum or urine. The major clinical features of these cases are schematically outlined in Figure 1. In eighty cases the clinical and pathologic features clearly satisfied the classic diagnostic criteria for multiple myeloma, that is, widespread osseous lesions comprised of abnormal plasma cells, with multiple fractures as the principal manifestation of the disease process were present. Anemia, hypercalcemia and renal damage figured prominently in this group and frequently were the critical factors leading to death. All but two of the eighty patients with this disease pattern were found to have a "characteristic myeloma protein abnormality" in the form of a serum and/or a urine electrophoretic spot. In two patients with "bony myeloma" in whom we could not demonstrate a serum or urine protein spot, a marked diminution of the normal gamma globulin fraction was observed. Summarizing this group of eighty cases of "plasma cell, bony myelomas," we can say that overproduction of an abnormal protein was found in seventy-eight cases, or 97 per cent.

A group of ten cases, considered to represent a closely related "lymphomatous variant" of myeloma, are outlined on the lower right of Figure 1. All ten patients were found to have "myeloma proteins," but in contrast to the first group of eighty cases the offending cell type in these cases was an abnormal lymphocyte rather than a plasma cell. The first two patients in this group presented all the clinical manifestations of bony myeloma, with widespread fractures, anemia and other characteristics, and, despite their lymphocytic cell type, showed neither the hepatosplenomegaly nor the lymphadenopathy which characterize the usual pattern of disseminated lymphosarcoma. We might therefore call these cases "clinical and chemical myeloma" although "histopathologic lymphosarcoma." The next six patients in this lymphocytic series exhibited characteristic lymphosarcomatous infiltrations of lymph nodes, liver and spleen, but the finding of "myeloma spots" distinguished them from the usual lymphoma pattern and indicated a biochemical relationship to the plasma cell disease. The last two cases outlined in this "lymphoma" group are essentially similar to the prior six except that these patients appear to have a particularly benign variant of this dis-

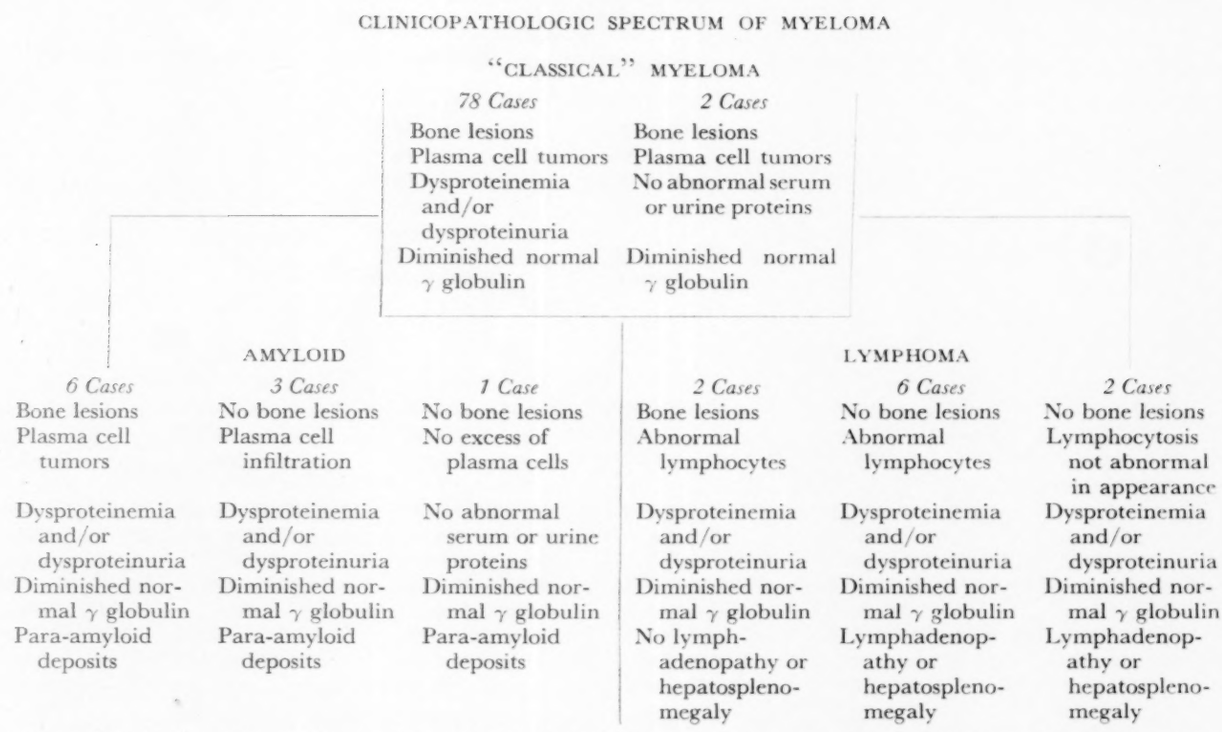

FIG. 1. Schematic outline of the clinical and pathologic features of 100 cases considered to fall within the "myeloma spectrum." The number of cases in which there is a particular disease pattern is indicated at the top of each grouping.

ease spectrum. Both of these patients have had generalized lymphadenopathy and hepatosplenomegaly for over five years. Repeated biopsy specimens have shown extensive infiltrations with "normal" lymphocytes. Without specific therapy, and without the aid of supportive measures such as transfusions or radiotherapy, both patients have remained in excellent health and at full functional capacity throughout this period. Obviously, it is impossible to state how long this quiescence will persist, and it is certainly possible that a more active and malignant disease pattern will eventually manifest itself.

If we agree that the production of abnormal proteins with unique physicochemical properties represents a specialized function of a particular cell family, we are probably justified in suggesting that these ten "lymphoma cases" are pathogenically related to the cases of plasma cell, bony myeloma. The plasma cell and the lymphocyte are believed to be closely related both in their origins and in their functional capacities. Both cell types have been demonstrated to participate in the synthesis of globulins in the normal state; it would seem not unreasonable that a lymphocytic proliferative process might on occasion be associated with a derangement of protein metabolism of the type commonly seen with plasma cell neoplasms.

Another clinicopathologic variant of the "myeloma spectrum" is the amyloid or para-amyloid phase. Schematically shown on the lower left of Figure 1 are the clinical and chemical features of ten patients who had symptoms and signs of varying severity referable to the deposition of para-amyloid material in various tissue areas. At this juncture we should clarify what is meant by para-amyloid. Para-amyloid refers to the amorphous proteinaceous material which is occasionally found in certain tissues and organs of patients who may or may not have obvious bony myeloma. The sites of predilection are the tongue, upper gastrointestinal tract, periarticular tissues and the heart. The liver, spleen and kidneys are less frequently involved than in the amyloidosis which is secondary to chronic suppurative disease. This latter "suppurative-amyloid" owes its name to the observation of its starch-like staining properties and its avidity for metachromatic dyes. The "myeloma-related substance," para-amyloid, which we are considering, has been repeatedly demonstrated to be much less consistently metachromatic than "suppurative-amyloid," and for this reason the

prefix "para-" was applied. Several observations within recent years have further delineated chemical differences between "myeloma para-amyloid" and "suppurative amyloid," and conceivably these may be completely unrelated processes.

Considering the ten patients shown on the lower left of Figure 1, it is to be noted that nine were found to have myeloma proteins. Of this group of nine patients with myeloma proteins, the first six exhibited bony myeloma along with their para-amyloid manifestations when this chart was prepared. In three cases the osseous symptoms antedated the para-amyloid signs and symptoms; in the other three the reverse sequence was observed, with para-amyloidosis first and bone destruction later. These six cases are readily acceptable as representatives of a common pathologic process despite this variation in course. The next three patients in this group (middle section, lower left, Fig. 1) showed only para-amyloid manifestations and no characteristic bone lesions when this chart was made. Myeloma proteins had been found and plasma cells demonstrated in abundance in marrow smears but skeletal x-rays revealed no lytic lesions. In recent weeks, one patient died of cardiac failure related to his cardiac para-amyloid; unfortunately permission for autopsy was not obtained. In another patient a pathologic vertebral fracture recently developed. Prior to the routine use of marrow aspiration, cases following the pattern of our patient who died of cardiac failure would have been classified as "primary amyloidosis" unrelated to myeloma para-amyloid. The second patient, with the pathologic fracture, would have been accepted as "myeloma para-amyloidosis." With the routine use of both marrow aspiration and electrophoresis it has become increasingly apparent that the majority of cases of so-called primary amyloidosis are on a plasma cell basis. We consider this para-amyloid material to be another abnormal protein product of malfunctioning plasma cells, but with the tendency to accumulate in certain predisposed areas of tissue. In some cases these deposits, if in vital areas, may prove lethal before the marrow collections of plasma cells become clinically apparent. These cases might more properly be designated primarily para-amyloidosis, with the understanding that abnormally functioning plasma cells are the probable source of this material.

We have encountered a single patient, outlined at the left center of Figure 1, with extensive para-amyloidosis, terminating in renal failure, in whom we could never demonstrate a myeloma serum or urine protein, nor could we find an excess of plasma cells in bone marrow sections. This case either represents a distinct and different pathologic entity, or conceivably was studied during a "burned-out" phase of the disease. It is of some interest that this patient's serum showed a marked reduction in gamma globulin comparable to that seen in many of the patients with myeloma with only a urine protein abnormality. Since this patient's urine was normal, however, the hypogammaglobulinemia by itself cannot be interpreted as a specific abnormality.

By way of summarizing the over-all clinical and pathologic picture presented thus far, we believe there is justification for expanding our concept of myeloma from the narrow restrictions of an exclusively bony disease caused by plasma cell proliferation, and to recognize that there may be a closely related lymphocytic proliferate disease on one end of this spectrum, and a para-amyloid variant at the other end. In the past, the trend has been to separate these patterns into distinct and isolated entities. We have chosen to consider the several patterns as variants of a common patho-physiologic process, and we believe that the transitional cases between the extremes at either end of the spectrum justify this grouping.

As stated at the outset, ours has been a principally "protein orientation," and we have frequently referred to characteristic myeloma proteins. Let us now proceed to define these characteristic abnormalities. Figure 2 shows a group of filter paper electrophoretic patterns obtained with a simple technic which was described a few years ago [*1*]. The qualitative changes in the serum proteins in this group of dysproteinemias are readily distinguishable one from the other. In the nephrotic syndrome we observe the characteristic elevation of an abnormal protein with a mobility intermediate between alpha-2 and beta globulins. In addition, there is a marked decrease in the albumin and gamma globulin fractions. The cirrhotic serum displays a diffuse and broad elevation of the gamma globulin fraction. The pattern labelled Hodgkin's disease was from a patient who not only had this neoplasm, which probably accounted for the elevation of the alpha-1 and alpha-2 fractions, but also was known to have posthepatitic cirrhosis,

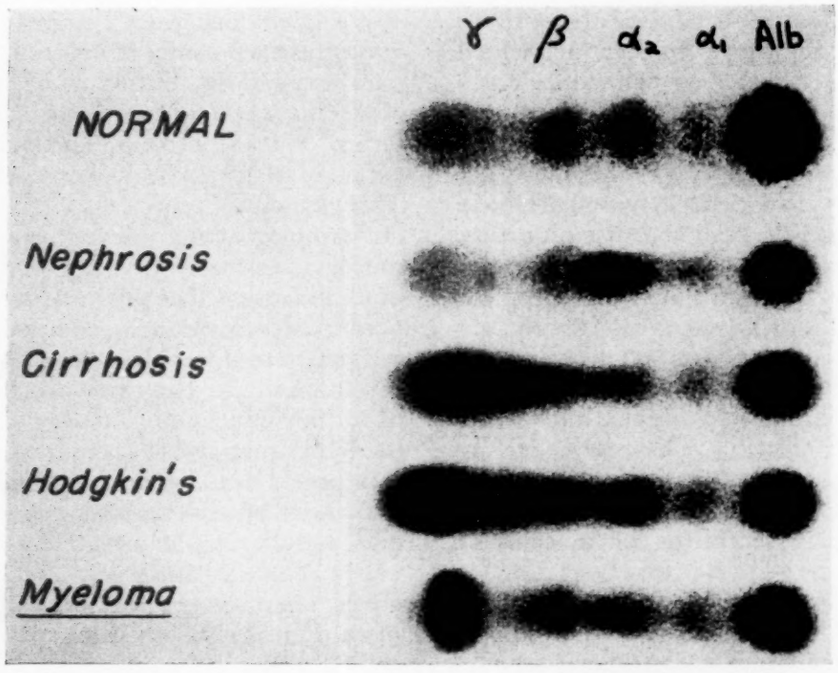

FIG. 2. Serum electrophoretic patterns in four disease states associated with protein abnormalities.

which was again reflected in a diffuse increase in gamma globulin. The discrete, electrophoretically homogeneous spot which is seen in the myeloma pattern is what we consider the characteristic myeloma abnormality. As Dr. Putnam will shortly point out, this spot can have, in the individual case, the electrophoretic mobility of a gamma or a beta globulin, but for a given case the abnormal protein retains the same electrophoretic mobility throughout the patient's course.

In Figure 3 there is a similar group of serum electrophoretic patterns obtained with a somewhat more elaborate filter paper system* which has the additional feature of quantitation of the several protein components. This is brought to your attention because this system is rapidly gaining favor as a routine laboratory procedure and has replaced chemical fractionation procedures, such as the Howe method, in the clinical chemistry laboratories of several hospitals (including our own) within the past two years. In this technic the filter paper patterns are scanned with a photodensitometer and the peaks are automatically plotted. These patterns are grossly similar to the patterns obtained with the moving boundary (Tiselius) system of free electrophoresis. Again, the electrophoretic homogeneity of the myeloma protein is quite readily distinguishable from the other pathologic protein derangements.

When the urine proteins of patients with myeloma are electrophoretically studied, another group of proteins of comparable electrophoretic homogeneity (Fig. 4). are observed. These are the so-called Bence Jones proteins and, to be sure, the majority satisfy the thermolability criteria for a positive Bence Jones test. It should be stressed, however, that the simple Bence Jones test as performed in most clinical laboratories is subject to frequent false negative and occasional false positive results. As a screening diagnostic procedure, we have found electrophoretic analysis of the urinary proteins more reliable than the Bence Jones test. In Figure 4 the serum and urine patterns of two patients are shown, one with myeloma, the other with the nephrotic syndrome. We again observe that the myeloma urinary protein is readily distinguishable by its homogeneity from the nephrotic urine pattern which shows essentially all the serum protein components.

Of the 100 cases referred to as falling within the myeloma spectrum, including the lymphoma and amyloid variants, in ninety-seven an abnormal spot was demonstrable in the serum and/or urine. The electrophoretic mobility of the

* Spinco Model R, Paper Electrophoresis System; Durrum-type. Beckman Instruments, Inc., Belmont, Calif.

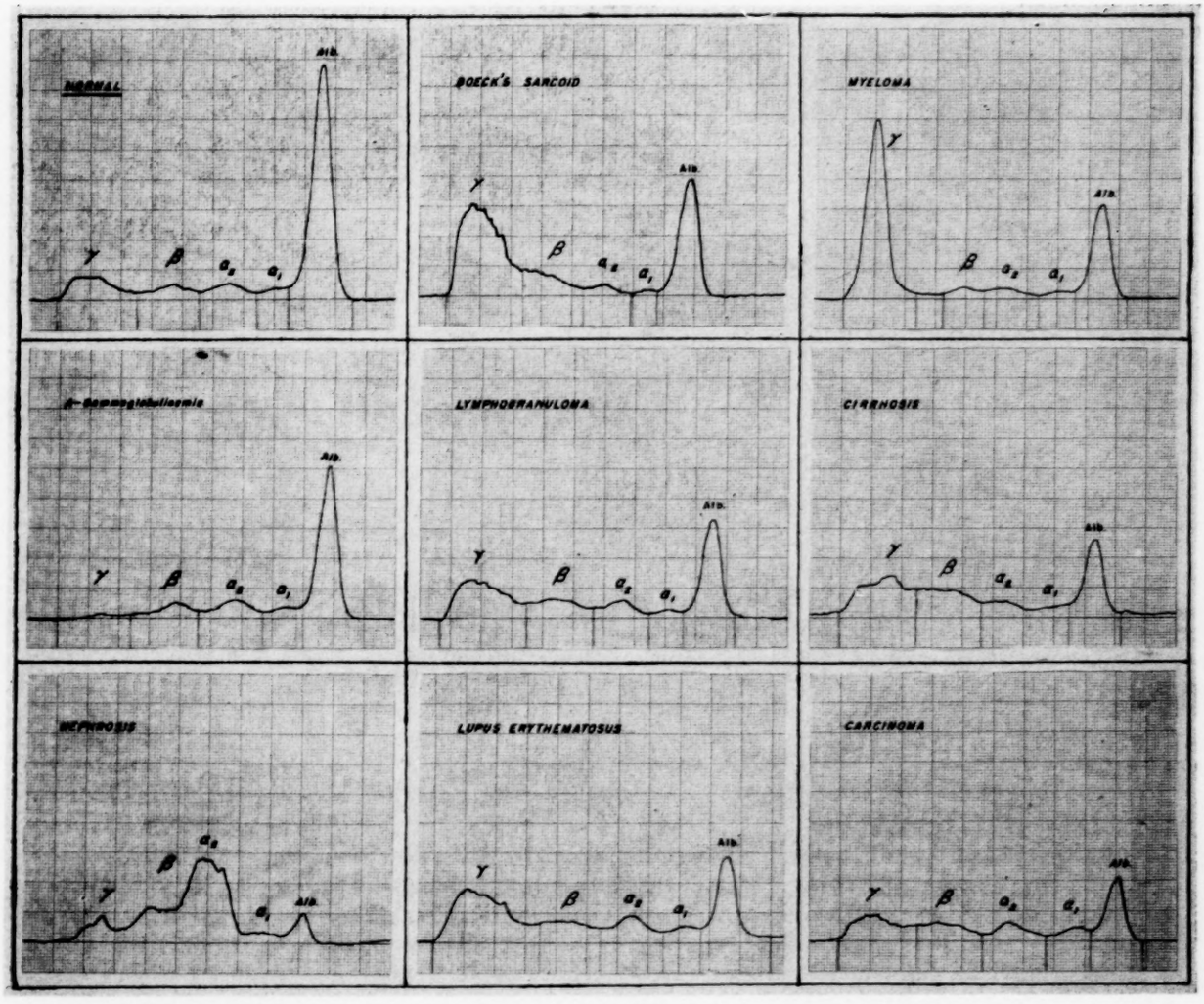

FIG. 3. Typical serum electrophoretic patterns from a group of patients with a variety of disease states associated with serum protein abnormalities. For reference, a normal serum pattern is shown in the upper left corner. These patterns were obtained with a recording densitometer from filter paper electrophoretic patterns. (Analytrol; Spinco Model R. Spinco Division. Beckman Instruments, Inc. Belmont, Calif.)

abnormal serum and urine proteins in each individual case is schematically presented in Figure 5. In forty-eight of these ninety-seven cases, both a serum and a urine abnormality was exhibited. In thirty-six, the serum pattern showed a spot, but the urine was either entirely negative or exhibited a non-specific type of proteinuria. In another thirteen cases no discrete peaks could be demonstrated in the serum but the urine was found to contain a characteristic abnormality. Thus, in approximately 97 per cent of cases a diagnostic abnormality can be shown if both the serum and the urine are studied by the electrophoretic technic.

As mentioned before, many patients without an abnormal spot will have a markedly diminished normal gamma globulin. This is also frequently apparent in those subjects whose abnormal peaks are in the range of fast gamma and beta mobility, and hence do not obscure this normal gamma region. The decreased capacity in patients with myeloma to form antibodies, which has been reported by Zinneman [2] and Lawson [3], is probably a reflection of this hypogammaglobulinemia. It may well be responsible for the apparent predisposition of many of these patients to infections, particularly recurrent bacterial pneumonias. Our patients with myeloma would seem to be suffering from "functional antibody poverty" in a land of "globulin plenty!"

I shall now turn this discussion over to Dr. Putnam who will discuss the physical chemistry of these myeloma proteins and some recent

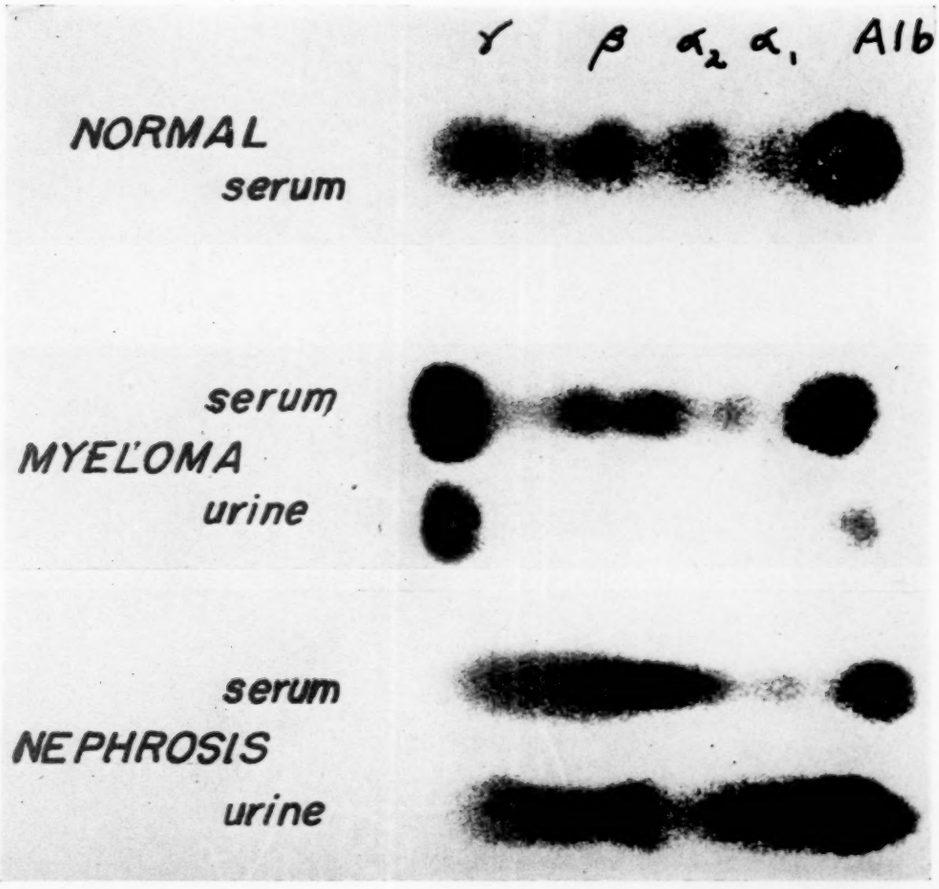

FIG. 4. Comparison of the serum and urine protein patterns of myeloma and nephrosis. The nephrotic urine is seen to contain all the protein constituents of the serum whereas the myeloma urine shows a large amount of homogeneous globulin and a small quantity of albumin.

isotope studies of their dynamics of formation and degradation. Dr. Putnam is Professor of Biochemistry at the new medical school of the University of Florida in Gainesville.

DR. FRANK W. PUTNAM: As Dr. Osserman has pointed out, multiple myeloma is of biochemical interest because the copious protein synthesis and the diverse nature of the proteins elaborated in this disease represent perhaps the most profound alteration in protein metabolism yet encountered.

This disease affects the plasma cells, which are probably implicated in antibody synthesis and in gamma globulin production. It results in the three possible types of aberration in protein metabolism already mentioned. The first of these is the formation of abnormal serum globulins; the second is the excretion of unique proteins in the urine, known as Bence Jones proteins and, as we shall indicate, the Bence Jones proteins are quite different from the abnormal proteins in the serum; and, thirdly but less frequently, there may be deposition of protein (para-amyloid) in the tissues.

The origin of the Bence Jones proteins is uncertain. It has been ascribed by some workers to the renal degradation of serum globulins or to a breakdown of tissue proteins; this is an hypothesis that we have put to test. Our approach to the study of the biochemical problems presented by multiple myeloma* has been threefold.

We first began with the purification and then with the physicochemical study of the abnormal

* The work described by Dr. Putnam was done in the Department of Biochemistry and in the Argonne Cancer Research Hospital of the University of Chicago and was aided by grants of the National Cancer Institute, National Institutes of Health, U. S. Public Health Service (C-1331-C4) and the American Cancer Society.

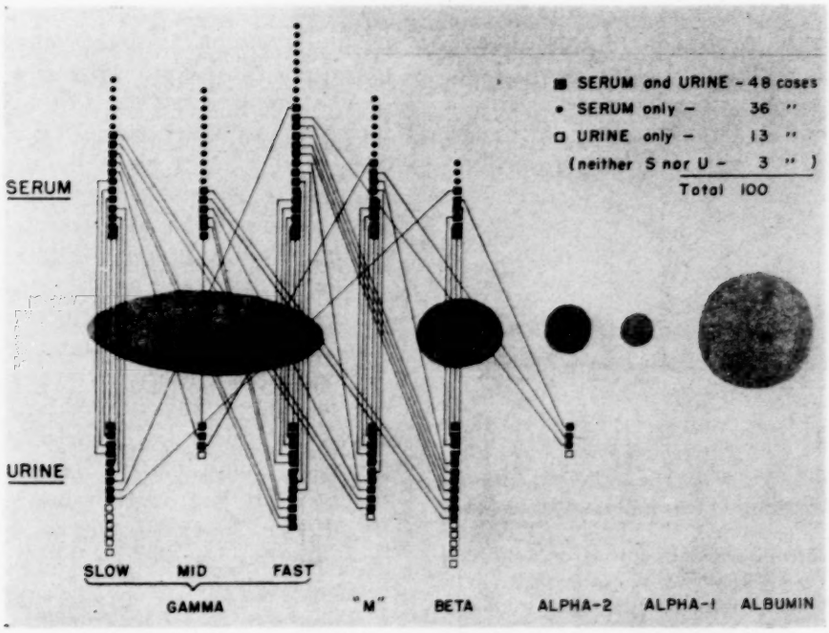

FIG. 5. Schematic distribution diagram of the electrophoretic mobilities of the abnormal serum and urine proteins in 97 of 100 cases of myeloma. The remaining three patients did not exhibit an abnormal constituent in either serum or urine. Unpublished data of OSSERMAN, E. F.

serum proteins and the urinary proteins, using the Tiselius electrophoresis apparatus and the analytic ultracentrifuge [*4,5*]. Secondly, we initiated a study of the chemical and structural characterization both of the abnormal globulins and of the urinary proteins [*6–8*]. These studies demonstrated quite clearly that different patients produced different types of abnormal serum globulins. Many of these abnormal globulins were related, both physically and, as Dr. Kabat will point out, also immunologically to the human normal gamma globulins. However, all the proteins studied could be differentiated from the normal proteins by some physical or chemical criterion. It appeared almost as if each patient made his own type of abnormal serum globulin. Similarly, the Bence Jones proteins proved to be almost individually characteristic and specific for the patient.

These methods, however, did fail to elucidate the relationships, if any, in the biosynthesis of the Bence Jones proteins and the abnormal serum globulins. They also failed to indicate whether or not there was a biosynthetic relationship in the formation of the abnormal proteins and of normal serum globulins. Thus, in the third phase of our work, which I shall also describe briefly, we undertook a study, employing isotopes, of the rates of synthesis of the abnormal globulins and also of the Bence Jones proteins [*9–11*].

One of the most perplexing and intriguing problems in this disease, to me as a biochemist, is the fact that, at first glance, no two patients appear to produce the same abnormal globulin or the same Bence Jones protein, and that a patient may have any one or two or even all three of these aberrations in protein metabolism. Today, we are going to give most of our emphasis to the question of the nature and origin of the abnormal serum globulins. From consideration of the physical and the chemical evidence that I shall discuss, and the immunologic evidence that Dr. Kabat will present, we should like an answer to the question of whether the myeloma globulin produced by a given patient is truly unnatural, truly abnormal, in the sense that it is formed only in the disease, or whether the myeloma globulin is produced selectively and profusely in the disease but is, in fact, a member of the family of normal gamma globulins, present physiologically only in minute amount.

As a first illustration of the unique type of proteins which are found in this disease, we can refer to the example of a crystalline cryoglobulin, that is, a protein which crystallized spontaneously upon cooling the serum of one patient.

This is one qualitative index of the uniqueness of some of the proteins produced in this disease, for normal gamma globulins have never been crystallized in the laboratory from the serum of any species of animal. Cryoglobulins, although infrequent in multiple myeloma, are most often found in this disease.

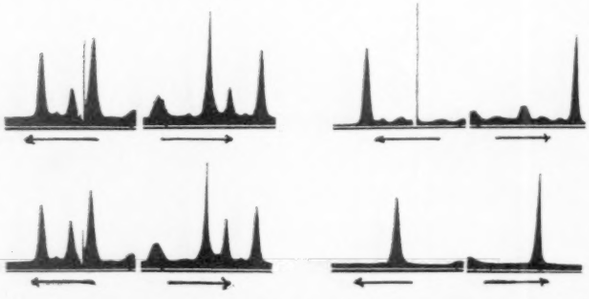

FIG. 6. Tiselius electrophoretic diagrams of a normal serum (upper right), a myeloma serum of the "beta" type (upper left), the urinary protein from the same myeloma patient (lower right), and a mixture of the serum and the urinary protein (lower left). Since all photographs were taken after migration for the same time at the same current, the distance of migration is comparable from diagram to diagram. Descending boundaries on the left, ascending boundaries on the right.

If we can turn our attention to the electrophoretic studies for a moment (Fig. 6), I should like to identify in these patterns the similarity with the paper-strip patterns that Dr. Osserman has presented. You will recall that since proteins are electrically charged, if an electrical field is imposed on a mixture of proteins, the proteins can be caused to separate to form individual boundaries yielding an electrophoretic pattern. In the Tiselius diagram, each peak represents a component. In the upper right of Figure 6 is the pattern for normal human serum at pH 8.6. The leading component is albumin, and is followed by alpha 1 and alpha 2 globulins, beta globulin and gamma globulin. An anomalous boundary remains at the origin. The sharp spike in the descending pattern is known as the beta anomaly. Its presence arises from a disturbance in the lipoproteins.

Because the experiment is performed in a U tube, there are two photographs in each panel. On the right is the ascending boundary, and on the left the descending boundary. These boundaries are obtained by the Schlieren optical method, and have this significance: Each peak represents a single protein component. Moreover, the area under each peak is proportional to the amount of protein present. Albumin comprises 60 per cent of the total protein in serum normally and has about 60 per cent of the area in the upper right hand diagrams.

A second significance is that if a single protein is present, a single boundary is obtained, as is the case in the lower right hand diagram, which happens to be the electrophoretic pattern of the urine of a patient who excreted a Bence Jones protein of quite homogeneous type.

Thirdly, the relative sharpness of these peaks is an indication of their electrical homogeneity. You will note that gamma globulin is quite diffuse in the pattern for normal serum. This is one of the first indications that gamma globulin may be comprised of a variety of closely related proteins which cannot be separated by this method. On the other hand, the boundary for the Bence Jones protein is quite sharp and is indicative of an electrically homogeneous protein.

There are many instances in disease wherein the serum electrophoretic pattern is changed; for example, infections and toxic conditions sometimes cause a 25 per cent increase in the gamma globulins [*12*]. Anything leading to antibody production will often cause a non-specific increase in gamma globulins and sometimes in beta globulins as well.

Then there are the dysproteinemias, such as cirrhosis of the liver, Boeck's sarcoid, lymphogranuloma venereum, and others, which cause marked hyperglobulinemia. These can be differentiated electrophoretically from multiple myeloma because in all these instances, one obtains a very diffuse and skewed boundary.

The significant thing in multiple myeloma, which makes these electrophoretic diagrams almost pathognomonic, is the characteristic presence of a predominant peak like a sharp spike that migrates somewhere in the globulin region. On the upper left of Figure 6 is an example of an electrophoretic diagram of the serum of a patient with multiple myeloma. In this case we observe between the beta and gamma globulins a predominant peak that corresponds, perhaps, to 45 per cent of the total protein. This component accounts for the entire hyperglobulinemia and is in the position where normally no peak occurs in the serum protein pattern. Again, I point to the sharpness of the peak as an indication of the homogeneity of the myeloma globulin. You might notice also the diminished gamma globulin in this case.

I want to make one other point from this

figure; namely, the Bence Jones protein excreted in the urine can readily be distinguished from the abnormal protein in the serum. The upper left diagram depicts the serum pattern of a patient who excreted the Bence Jones protein in the urine, as shown in the lower right diagrams. Electrophoretically, the Bence Jones protein migrates in the alpha 2 position if we add it back to the serum. Electrophoretically, then, the two pathologic proteins are different.* Moreover, the Bence Jones protein has a molecular weight about one-quarter that of the abnormal serum globulin and, of course, only the urinary protein gives the characteristic heat coagulation test. This is a representative pattern but not the only characteristic pattern for multiple myeloma serum for, as a matter of fact, a variety of bizarre and unpredictable electrophoretic patterns are obtained in multiple myeloma.

A series of representative myeloma serum diagrams are depicted in Figure 7. In five of six cases we see the common characteristic of a sharp, homogeneous spike migrating somewhere in the globulin region. You will recall that Dr. Osserman stated that the paper electrophoresis pattern of the serum of a given patient remains specific throughout his rather short life span, changing, perhaps, only in the total amount of the abnormal globulin. Similarly, the Tiselius patterns remain specific for the individual but, as you see, are quite different from one serum to another. In serum No. 2, the abnormal globulin migrates more slowly than gamma globulin; in No. 6, it has the mobility of gamma; and in No. 17, it is intermediate between beta and gamma globulins. Yet in serum No. 20, the pathologic protein migrates even more rapidly than beta globulin. Another interesting observation is the fact that many of these patients almost have an agammaglobulinemia. For example, in serum No. 20 you will note that with the synthesis of the abnormal serum globulin there is virtual suppression of the formation of all the other globulins. It is quite perplexing that such different patterns should be given by different patients. Moreover, it appears that approximately 20 or 25 per cent of patients do not have hyperglobulinemia. As an example, note the absence of a sharp peak in the pattern for serum No. 21, which has a pattern that is relatively similar to that of normal serum.

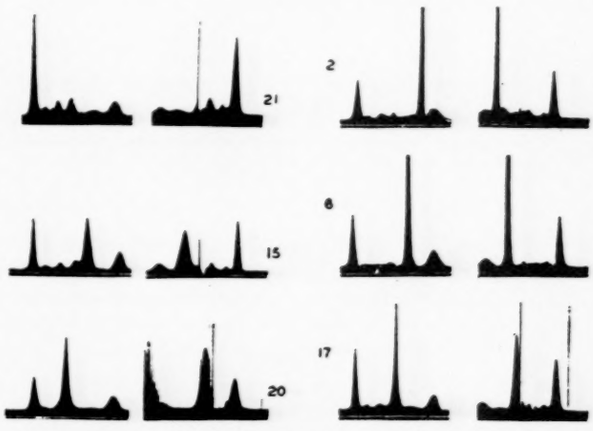

FIG. 7. Representative Tiselius electrophoretic diagrams of the serums of six patients with multiple myeloma. Ascending boundaries on the left; descending on the right.

* Editor's note: This experiment oversimplifies a rather complex situation, and may therefore be somewhat misleading. On this account, Dr. Osserman was asked to summarize his own experience in this connection, and to give a fuller description of the phenomenon:

In an electrophoretic study of the urinary proteins of 100 patients with multiple myeloma 61 per cent were found to have abnormal urinary protein peaks. Of these sixty-one patients with proteinuria, in thirteen the serum failed to show any characteristic abnormality. In twenty-six cases a serum peak of identical mobility with that found in the urine was demonstrable (group I) and in virtually all these cases the proteinuria consisted of a mixture of albumin and the alpha and beta globulins, in addition to the myeloma spike. In twenty-two cases (group II) the myeloma urinary protein was found to have a different electrophoretic mobility than the serum peak (U faster than S in nineteen cases; U slower than S in three cases). Dr. Putnam's case (Fig. 6) is representative of this group, and certainly the evidence is convincing that these serum and urine proteins are separate and distinct constituents. In the group I cases, however, in which the serum and urinary proteins are of identical mobility, it would appear that a more extensive renal defect is present, and larger protein molecules (including the abnormal myeloma globulin) are being lost in the urine. As Dr. Putnam indicates, in a group II case addition of the urine protein to the serum will result in an electrophoretic pattern with two distinct pathologic peaks. In the group I cases, however, this maneuver results in superimposition of the abnormal urine peak upon the abnormal serum peak.

It is noteworthy that the urines from patients in group I are almost always negative for Bence Jones protein as opposed to the majority of the urine from patients in group II which are positive for Bence Jones protein. Further evidence of the qualitative differences between the proteinuria of the group I and II cases is obtained from the results of carbohydrate analysis [7] of the abnormal urinary peaks. In the group I cases, showing proteins of identical mobilities in serum and urine, both of these abnormal peaks are Schiff-positive. In the group II cases the abnormal serum proteins are Schiff-positive, but the urinary peaks are Schiff-negative and apparently devoid of conjugated carbohydrate.

I think, then, it can be demonstrated that there is a wide range in the electrical mobilities of the abnormal globulins of multiple myeloma serums. This indicates that there are electrical differences in the globulins in different patients. We have already published [4] a histogram of the

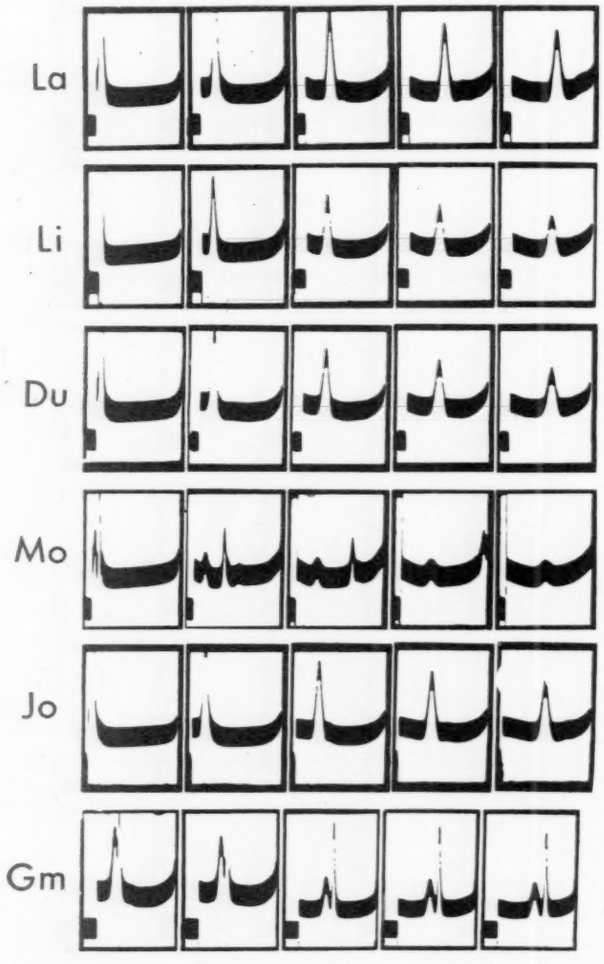

FIG. 8. Ultracentrifuge diagrams of the purified myeloma globulins from the serums of various patients. The rotor speed was 59,780 r.p.m. with photographs at eight or sixteen minute intervals. Sedimentation proceeds to the right in the photographs.

mobility range of the abnormal globulins from the serums of approximately eighty patients, and have shown that the mobility range is approximately tenfold. However, there was a bimodal distribution. A great many of the myeloma globulins have mobilities that cluster in the region of gamma globulin, and there is another maximum in the region which has a mobility just less than that of beta globulin. Dr. Kabat will probably review the immunologic relationship of what we would call the "gamma" type of myeloma globulin with the normal protein and, similarly, for the "beta" type.

Although there is a wide variation in the electrical properties of the myeloma globulins, most of these abnormal proteins have similar molecular kinetic properties such as sedimentation rate in the analytic ultracentrifuge [4]. In some respects, analysis with the ultracentrifuge is quite similar to electrophoretic analysis by the Tiselius method. One can determine the homogeneity and the molecular weight, or at least a function of the molecular weight, by use of the analytic ultracentrifuge. The Tiselius apparatus gives a constant known as the mobility which can be measured under defined conditions and is a function of the ionic charge of the protein. The analytic ultracentrifuge also gives a constant, known as the sedimentation constant; the sedimentation constant, however, is a function of the molecular size and shape. By optical methods quite similar to those used in the Tiselius apparatus one can get sedimentation patterns for the serums, but the patterns that I present are actually for the purified multiple myeloma globulin from individual patients.

For example, at the top of Figure 8, we see a series of diagrams, taken at five intervals, of the sedimentation in the analytic ultracentrifuge of a purified myeloma globulin from patient La. There is a single sharp sedimenting boundary, indicating molecular homogeneity. In this case the sedimentation constant is 6.6 Svedberg units. This corresponds to a molecular weight of 160,000, a value identical with the molecular weight of normal gamma globulin. The majority of the proteins we find in multiple myeloma have such a sedimentation constant and have molecular weights analogous to those of the normal protein.

However, occasionally we encounter proteins with unusual sedimentation properties, proteins designated as macroglobulins. These are found in a syndrome known as Waldenström's macroglobulinemia, in which the biochemical change is the fact that there is a sharp sedimenting component which has a higher molecular weight and a sedimentation constant of 16 or 24 Svedberg units; in fact, there may be several such components. For example, the myeloma globulin of patient Mo contains three peaks. The slowest moving component (the peak at the left in each diagram) corresponds to a globulin of normal size. However, the major sharp boundary represents a component which has an average molecular weight of about 1,000,000. Another

instance in which there is a sharp, rapidly sedimenting component is seen in the diagrams for Gm. In this case the globulin has a molecular weight of the order of 400,000. To be sure, such high molecular weight components are present in normal serum but only in minute amount, whereas in this instance they predominate among the globulins of the serum.

These representative figures illustrate the results of the physical studies we have made so far. It was soon apparent that certain differentiations could be made by physical methods, but we were also interested in making a structural characterization of these proteins to ascertain if they were structurally or chemically related to the normal gamma globulin. This could be done by means of a method known as the amino end group method.

If you recall the structure of a protein, typically, it is a series of repeating residues —NC, CH, CO— bound together by the peptide bond and having side chains which can be any one of eighteen or twenty-odd amino acids. The number of amino acid residues in the case of gamma globulin would be 1,300, that is, there are about 1,300 amino acids of approximately eighteen different types in the molecule of gamma globulin. However, if the polypeptide chain is open, it must have a beginning and an end. The beginning would have a free alpha amino group, just as the other end of the chain would have a free carboxyl group. We call the initial amino acid that has a free amino group, the N-terminal amino acid. We can identify it by coupling the alpha amino group with the reagent developed by Sanger [*13*], fluorodinitrobenzene. Not only can we determine the nature of this group, we can also estimate it quantitatively and thus can determine the number of polypeptide chains. This number should be integral; that is, there should be integral numbers of amino end groups, each corresponding to a polypeptide chain. Fractional or non-stoichiometric amounts would indicate heterogeneity. Thus we also have a measure of chemical homogeneity.

This method of analysis, the amino end group analysis, was applied to the study of normal human gamma globulin by Emil Smith and myself, independently [*6,14*]. The first observation to be made was that there is approximately 1 mole of N-terminal aspartic acid and approximately 1 mole of N-terminal glutamic acid per mole of protein. Normal gamma globulin appeared to consist of one chain that began with aspartic acid and another chain that began with glutamic acid, and there were perhaps 700 amino acids in each chain. However, a closer inspection of the data revealed that when we analyzed different sub-fractions of the gamma globulin, the ratio of the amino end groups varied from unity to almost twice unity.

There is no way in which one could explain such variations other than by concluding that there must be more than one kind of gamma globulin present in normal pooled human gamma globulin. For example, one type could be a gamma globulin which began with the aspartic acid and another a protein which began with glutamic acid.

I should like to differentiate clearly between proteins, for example, in which the first amino acid is aspartic acid or the first amino acid is glutamic acid. We might have a protein with two peptide chains, each beginning with aspartic acid, another one with two chains each beginning with glutamic acid and so forth. That appears to be the situation for normal pooled human gamma globulin. For the globulins of other species the picture is even more complex, except in the case of the rabbit.

When this type of analysis was applied to the purified globulins in the serums of twenty patients with myeloma there were about five major types of proteins that we encountered [*15*]. All these twenty patients' proteins proved to be different from normal pooled human gamma globulin, either in the number or in the nature of the amino acid end group. Five major types are listed in Table 1. The first group comprising proteins La and Li we can call N-leucyl globulins, because these molecules have two end groups of leucine, and they contain only small amounts of aspartic acid or glutamic acid as an end group. This could be attributed to the presence of normal gamma globulin because, of course, there was no way that we could remove the normal protein from the myeloma globulin, if both had a mobility in the same range. Now, leucine has not been detected as an end group in normal pooled human gamma globulin so it might be considered that, possibly, these leucine globulins are truly unnatural.

Then, the second group—and there are five proteins listed—we can call N-aspartyl globulins, because these proteins all have two moles of N-terminal aspartic acid, as if they had two chains each beginning with aspartic acid. These

TABLE 1

N-TERMINAL GROUPS OF MYELOMA GLOBULINS

	Protein	pI	Mobility (pH 8.6)	S_{20} (S)	Asp (moles per 160,000 gm.)	Glu (moles per 160,000 gm.)	Leu (moles per 160,000 gm.)	Ala (moles per 160,000 gm.)	Ser (moles per 160,000 gm.)
Type I	La	>6.7	−0.67	6.4	0.17	0.25	2.00*	....	....
	Li	6.7	−0.76	6.2	0.04	0.08	2.02	....	0.15
Type II	Th(cryoglobulin)	7.5	−1.1	6.6	1.80	0.16	.....	....	....
	AG(cryoglobulin)	>6.4	−1.1	6.6	2.04	.....	.....	....	....
	Mi(cryoglobulin)	.....	−1.3	6.7	2.06	0.16	.....	....	....
	Wi	~6.6	−1.1	7.0	1.80	0.12	.....	....	....
	Men	.....	−2.1	6.3	2.32	.....	.....	....	0.17
Type III	R(cryoglobulin)	.....	insol.	7.6†, 11	2.00	2.64	.....	....	....
	Gu(cryoglobulin)	.....	insol.	6.0	2.29	2.21	.....	....	....
	Jo	<6.7	−2.4	6.1†, 9	2.05	2.65	.....	....	0.07
Type IV	Se(cryoglobulin)	.....	−0.8	6.4	.....	2.65	.....	....	....
	I(cryoglobulin)	.....	insol.	7.6†, 11	0.15	2.77	.....	....	....
	McF(cryoglobulin)	5.5	.....	7.18†, 28	0.12	3.10	.....	....	....
	Win	.....	−1.56	6.1	trace	3.87	.....	....	....
	Kl	.....	−1.9	.....	0.11	5.63	.....	....	....
Type V	We	.....	−0.96‡	6.23	trace	1.52§	0.23	0.20	0.32
	Du	6.7	−1.18‡	6.2	0.49	0.50	.....	0.66	....
	Mo	.....	−1.82‡	6.2, 16†, 24	1.75	0.42	.....	....	0.34
	Gm	≪6.7	−2.39	6.2†, 8.8†, 12	1.26	1.67	.....	....	0.12

* Contains an unidentified N-terminal amino acid besides leucine.
† Signifies major component.
‡ Signifies heterogeneous in electrophoresis.
§ Contains 1.0 mole of an unidentified N-terminal amino acid.

proteins are devoid of glutamic acid in that position or else they have only small amounts. Again, the fractional figure could represent a small amount of the normal globulin present.

We have also determined the physical constants of these proteins but I shall not go into the data, which are listed in Table 1. You might notice that most of the aspartic globulins have the normal sedimentation constant of 6.6 Svedberg units, which would correspond to a molecular weight of 160,000. Many of the proteins have a mobility in the range of gamma globulin, but protein Men has a higher mobility.

Then, in a third group of myeloma globulins we found proteins with both types of end groups, aspartic and glutamic acid, but in twice the normal amount. Some of these are rather unusual proteins in a number of other respects; for example, two of these proteins (R and Gu) were cryoglobulins; that is, they crystallized or gelled spontaneously when the serum was cooled.

A fourth type of protein, of which we encountered quite a number of representatives, we could call N-glutamyl globulins. There are five glutamyl globulins listed in the table. These proteins are either devoid of aspartic acid in the amino end group or have only small amounts. Some of the glutamyl globulins have 3 moles of end groups, denoting the presence of three peptide chains each beginning with glutamic acid. Apparently, globulin Win has four chains, and the others may have five or six.

There is a fifth group listed, which is heterogeneous both in physical-chemical analysis and in amino end group analysis. I shall not go into them but I might mention that recently we studied a sample of myeloma globulin obtained from Dr. Osserman. This protein differed from all those listed in that it had both glutamic acid and phenylalanine in the N-terminal position. Of course, phenylalanine has not been detected in the N-terminal position in normal human serum globulins. This discussion does not exhaust the list of possibilities, because Emil Smith and others have recently published a paper describing similar studies of four myeloma globulins, each of which differed from the other, and three of which differed from any that are listed in Table 1 [*16*].

Included in the data on the twenty myeloma globulins are the results of studies on eight cryoglobulins, rather unique and quite rare proteins. All the cryoglobulins differ from normal, pooled gamma globulin by several physical and chemical criteria, such as the amino end group. Moreover, seven of these cryoglobulins differed from the others. There were only two of these unique globulins that were alike in all the properties we investigated.

It may be that all seven of these cryoglobulins are, in fact, present in minute amounts in the serum of each of us, but there is a more likely interpretation, namely, that these globulins are, in fact, unnatural proteins, formed only in certain disease states particularly in myeloma. This latter hypothesis does not deny the possibility that these abnormal proteins may exhibit certain of the physical and immunologic characteristics of normal gamma globulins.

We have made a similar extensive analysis of the Bence Jones proteins, the proteins that appear in the urine and have molecular weights of the order of 40,000 to 44,000. I shall not go into the details, except to say that recently we have done some collaborative work with Dr. Leonard Korngold of the Sloan-Kettering Institute which has thrown new light on the nature of these proteins. We had published data which indicated that in the study of the Bence Jones proteins from eight patients each of these proteins could be differentiated from the others by one or more criteria, as if each patient excreted his own unique Bence Jones protein [*7*]. In the collaborative study with Dr. Korngold we found some relationships in properties making possible a grouping of the Bence Jones proteins. For example, the proteins that Dr. Korngold found to be of his antigenic type A all had essentially the same sedimentation constant, about 3.4 Svedberg units, and they were all devoid of aspartic acid as an amino end group. However, the proteins were of different types. For example, the isoelectric points varied from pH 4.6 to 6.7. Correspondingly, there was considerable variation in the mobilities. Although there were eight proteins of antigenic type A, Dr. Korngold believes that they can be divided into sub-types by the serologic method. For our part, we observed that whereas the end groups of many of the proteins of antigenic type A could not be characterized by the Sanger method of analysis, one specimen had tyrosine and another had isoleucine as amino end groups.

Then, there were four proteins that were in Dr. Korngold's antigenic type B. All these had aspartic acid in the N-terminal position—between 1 and 2 moles per 44,000 gm. of protein. However, the antigenic type B proteins differed somewhat in their mobilities, and they also fell into two size classes. Two proteins had sedimentation constants that correspond to a molecular weight of about 20,000 or 25,000, and two appeared to have a size of about 40,000 to 44,000.

That was the situation, then, when we undertook isotopic studies of protein synthesis in patients with multiple myeloma. It appeared that a variety of proteins could be formed by patients with this disease; each patient perhaps making an abnormal serum globulin or excreting a unique Bence Jones protein or even having both defects in protein metabolism.

Some time ago, the question naturally arose as to whether or not there was any precursor relationship in the formation of these two types of abnormal proteins. We have performed a number of experiments [*9–11*] in which patients were given isotopically labeled amino acids, the tracer being the stable isotopes N^{15} and C^{13} or radioactive carbon, C^{14}. I have selected just one example from our recent studies to illustrate the type of approach.

The experiment was performed in the following fashion: A patient was chosen who excreted a Bence Jones protein in the urine, and who had an abnormal globulin in the serum of the gamma type. He was given an intravenous injection of 450 microcuries of DL-glutamic acid, which was labeled in the number one carbon, that is in the alpha carboxyl position. This particular position

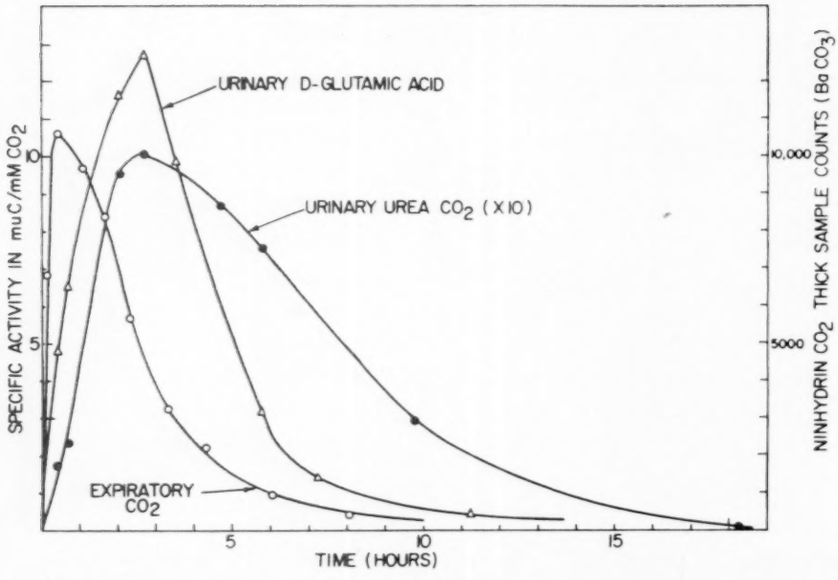

FIG. 9. Specific activity of the expiratory CO_2 (left ordinate) and of the CO_2 released from urinary amino acids by ninhydrin (right ordinate) and from urinary urea by urease (left ordinate) in samples obtained from a patient with multiple myeloma injected with 450 μg. of DL-glutamic acid 1-C^{14} at zero time.

was chosen because it is accessible to decarboxylation by ninhydrin, in which case both the D and L forms react. Moreover, the L form reacts specifically to release the radioactive carbon upon action of the optically specific glutamic acid decarboxylase that can be obtained from E. coli.

The patient was fitted with a venous catheter and a urethral catheter, and frequent blood and urine samples were taken. Indeed, we obtained about seventy urine samples over a period of approximately four days and twenty blood samples during a period of about twenty-four hours. The expiratory CO_2 was also collected for measurement of its radioactivity. Then, from each blood sample the globulin was isolated in a purified form and was characterized by electrophoresis. Similarly, the Bence Jones protein was isolated from the urine of each of the samples.

This experiment also gave us the first opportunity to follow the fate of D-glutamic acid in man. As one might expect from a knowledge of the labeling in this amino acid and of its fate in the tricarboxylic acid cycle, there was a rapid increase in the specific activity of the expiratory CO_2. As illustrated in Figure 9, the C^{14} content of the expiratory CO_2 attained a maximum approximately twenty-five minutes after the injection. Thereafter it declined rapidly, with a half-time of about eighty-five minutes. In other words, this is just a reflection of the rapid metabolism of L-glutamic acid, and, correspondingly, there should be a rapid decline in the activity of the serum glutamic acid. The same carbon, the respiratory CO_2, which appears in the expiratory CO_2, also appears in urea. Hence we had the opportunity to measure the decrease in radioactivity of the urinary urea carbon, which was obtained by reaction with urease. We found maximum specific activity of the urea carbon at two and a half hours, and then there was a rapid decline.

Just as a little side feature, we measured the excretion of the urinary amino acids and found that the maximum activity of the excreted urinary amino acids was at two and a half hours, after which there was a rapid decline. It has been suggested [*17*], and this, perhaps, may be quite true in the rat, that D-glutamic acid is excreted as the metabolite known as pyrollidone carboxylic acid. We believe this was D-glutamic acid because it reacts with ninhydrin but does not react appreciably with the enzyme.

A long time ago Kögl [*18*] suggested that D-glutamic amino acid appears in tumor proteins but we were not able to demonstrate significant amounts in the Bence Jones protein which was obtained from the urine of this patient.

To recapitulate, the labeled amino acid was

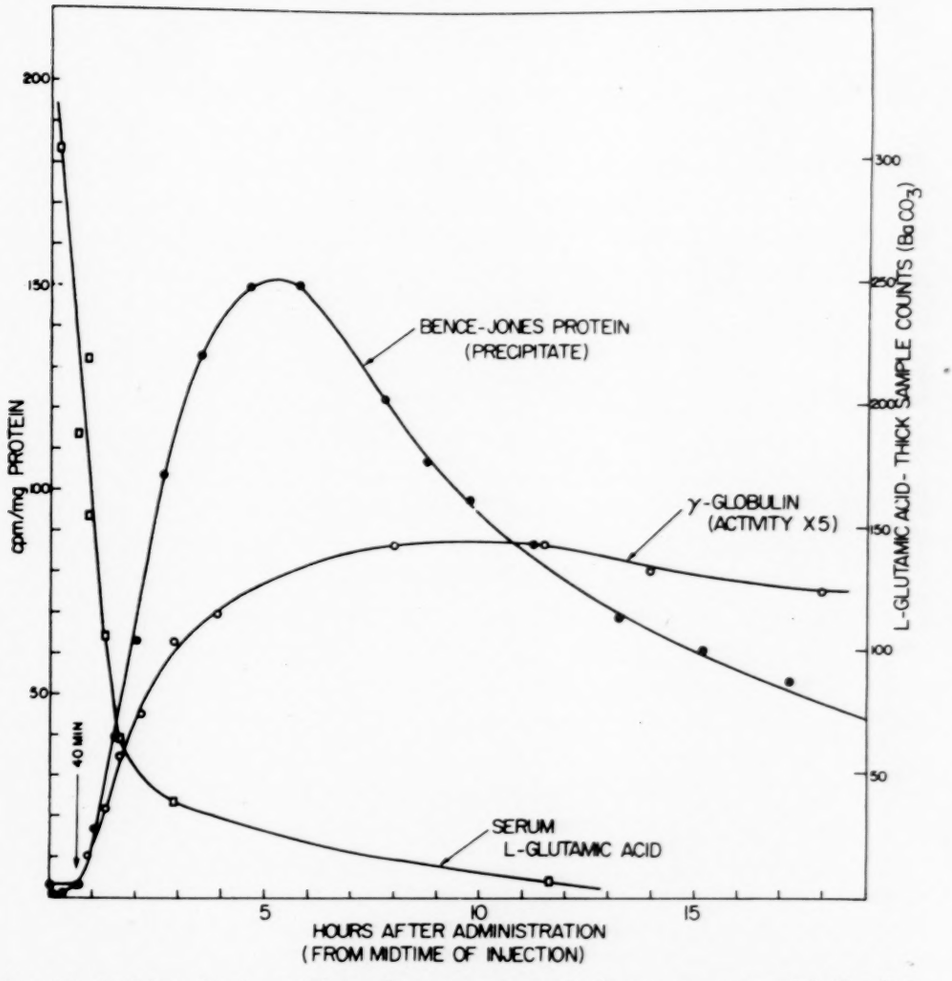

FIG. 10. Activity of urinary Bence Jones protein and of serum gamma globulin (left ordinate) and of serum L-glutamic acid (right ordinate) from the patient referred to in the text and Figure 9.

injected into the patient, and twenty-five minutes afterwards there was a maximum in activity in his expiratory CO_2, and two and a half hours afterwards there was a maximum in his urea CO_2. What was the rate of incorporation of C^{14} into the proteins synthesized by this patient?

As one might have anticipated from the CO_2 curve, there was an extremely rapid decline in the activity of the serum L-glutamic acid (Fig. 10), but during the first forty minutes no radioactive protein was detected either in the serum or in the urine, although a number of samples were analyzed. This period we could call the transit time. We define the transit time as the sum of the time it takes for the body to form Bence Jones protein or gamma globulin, for this protein to be released into circulation and, in the case of the Bence Jones protein, for the protein to be excreted in the urine.

Let us now consider the Bence Jones protein. You will note that after the transit time there was a rapid increase in the radioactivity of this protein, so that it reached a maximum at about five and a half hours, in other words, only a few hours after the maximum in the activity of the urea. Hence, the C^{14} of the glutamic acid was excreted almost as fast by way of incorporation in the Bence Jones protein as it was by way of metabolism and elimination as urea carbon. After the maximum was attained there was an extremely rapid decline in the radioactivity both of urea and of the Bence Jones protein.

From the balance isotopic data in another experiment [*11*] we have been able to show that the bulk of the Bence Jones protein formed at

any given interval of time is excreted by the body in the ensuing twelve hours. This, then, is one of the clearest indications that the Bence Jones protein cannot be a degradation product of tissue breakdown.

The question naturally arises, is it possible that the Bence Jones protein is formed by a breakdown of the serum gamma globulin? If you examine this curve you will observe that the gamma globulin began to have radioactivity after approximately forty minutes, but its maximum occurred at ten hours; subsequently its activity declined more slowly than that of the Bence Jones protein. I should say, it declined in a manner corresponding to the turnover curves usually found for serum proteins, which vary from five to twenty days according to the species and the investigator.

A study of these data would indicate to one acquainted with the analysis of isotopic rate data that the Bence Jones protein could not be formed from the gamma globulin. If we had a homogeneous system, which, of course, we do not have here when we are dealing with a patient's serum as compared with the urine, and if there were a material *A*, which was the sole and the immediate precursor of *B*, then we could expect, if we gave a labeled amino acid which formed protein A, that protein A would shortly begin to receive radioactivity. Its activity would reach a maximum and then would decline over a period of time. If A were, in fact, the sole and immediate precursor of B, then B, of course, would be labeled but as of a somewhat later time. The activity of B would reach a maximum which would intersect somewhere on the declining portion of the curve for A, and then B also would begin to fall off in activity; so for A to be the precursor of B, A has to be of higher activity at some point and it has to be prior to the activity maximum in B.

If we look at Figure 10, we see that the gamma globulin cannot have been the precursor of the Bence Jones protein, for the gamma globulin does not have a higher initial activity than the Bence Jones protein. In fact, the gamma globulin has a much lower activity (its curve has been multiplied by five for illustrative purposes). In other words, the myeloma globulin has a lower activity than the Bence Jones protein initially, and its maximum activity occurs at a later time. Hence, the serum gamma globulin cannot have been the precursor of the Bence Jones protein.

Is it possible that the Bence Jones protein was a precursor of the gamma globulin? Well, as plotted in this figure this hypothesis might appear to be a possibility because of the fact that, as plotted, the maximum activity of the serum globulin does appear to intersect the declining portion of the curve for the Bence Jones protein, or at least the maximum comes fairly close to the declining portion of the curve. But this is really an illustrative trick, for we do not have a homogeneous system. We are sampling urine in one case and we are sampling serum in another case.

From our data on the rapidity of the excretion of the Bence Jones protein it is clear that this protein is handled almost wholly as a nitrogenous excretory product. On the other hand, the serum globulins which are synthesized are diluted by the pool of serum globulins in the body. Now, one has to make some kind of estimate of this dilution. Perhaps it is fivefold, perhaps it is tenfold. I should say, therefore, that the interpretation of this experiment and of similar isotopic experiments is as follows:

The Bence Jones protein is not formed by degradation of tissue proteins or of serum proteins but, rather, it is formed *de novo* by synthesis from the ingested amino acid. The Bence Jones protein, once formed, is rapidly eliminated from the body. This does contribute to some of the metabolic features of the disease, because it represents a considerable diversion of the nitrogen from the body. Some patients excrete up to one-third or more of their daily nitrogen intake by way of a protein which has no known metabolic function or normal counterpart. In some cases, then, perhaps one-third of the ingested nitrogen is excreted by way of this protein, which is rapidly formed and rapidly eliminated.

Our present data, although they exclude the possibility of the serum protein or of tissue proteins being a precursor of Bence Jones protein, are compatible with but certainly do not prove the hypothesis that the Bence Jones protein might be on the route to synthesis of normal proteins. We do believe that in our work somewhere we have to find a place for the Bence Jones protein, and that further experiments have to be devised.

DR. OSSERMAN: Despite the vast amount of work which has been done in this field, it is apparent that several fundamental problems remain unresolved. The experimental evidence [*19–21*] that the myeloma cells are truly the

origin of these serum and urine proteins is quite convincing, but the relationship which these proteins, particularly the serum constituents, bear to the normal gamma globulin fraction is still a matter of considerable conjecture and controversy. In several diseases in which there is a "diffuse" elevation of gamma globulin, such as cirrhosis and the collagen disorders, an increase in the number of plasma cells in the bone marrow can be and has been documented. This diffuse type of increase indicates that many different proteins of similar but not identical electrophoretic mobility are in overproduction. It has been suggested that some as yet obscure stimulus arising from the pathologic process is operating on all the plasma cells in these disorders and all the normal gamma globulins are accordingly produced in excess. The overproduction of a single, homogeneous protein constituent in myeloma suggests that in this pathologic state a single plasma cell has become malignant, and in so doing has retained its globulin-forming function with resultant overproduction of a single "normal gamma globulin." While the end terminal amino acid studies, to which Dr. Putnam has referred, suggest that there may be real chemical and structural differences between isolated myeloma globulins and the normal gamma fractions, we must keep in mind that the normal gamma fractions used as reference standards are heterogenous and the terminal amino acid data consequently reflect mean values for this family of proteins.

Even if we could provide a definitive answer to the question of normalcy or abnormalcy of these myeloma serum globulins, we would still be left with a vast number of unsolved problems with respect to the urinary Bence Jones constituents. As Dr. Putnam has indicated, these urinary proteins are of considerably smaller molecular size than the serum globulins—of the order of molecular weight of 40,000, or approximately one-fourth the size of the serum constituents. The isotope experiments to which Dr. Putnam referred have provided strong evidence that the Bence Jones proteins are not directly derived from these serum globulins and it would appear that they are independently elaborated. It seems reasonable to suppose that the neoplastic plasma cells form two separate protein constituents, and that our failure to find the Bence Jones protein "spikes" in the serum may be due to the rapidity with which these smaller molecular components are excreted by the kidneys. By means of precise immunochemical technic, several investigators have presented convincing evidence that these Bence Jones proteins are in truth present in minute amounts in the serum of many patients with myeloma. Dr. Elvin Kabat, with Drs. Gutman and Moore, was among the first to work on this problem over twelve years ago. Their findings have stimulated a large number of subsequent investigators to pursue the immunochemical approach to the problem of the myeloma proteins. Although Dr. Kabat has since left this area and gone on to other investigative pursuits, we have asked him to come out of his twelve year silence on myeloma protein problems and provide us with a critical review and appraisal of the large body of immunochemical data on the myeloma proteins which has accumulated over the past several years. Dr. Kabat is Professor of Microbiology assigned to the Department of Neurology.

DR. ELVIN A. KABAT: Dr. Putnam and Dr. Osserman have already indicated the enormous complexity of the problem with respect to the serum and urinary proteins in myeloma. I do not believe that any two myeloma proteins thus far characterized have been shown to be identical by all criteria, so we must face the possibility that there may be as many as a hundred or more different myeloma serum and urine proteins.

A long while ago, Bayne-Jones and Wilson [*22*] studied Bence Jones proteins immunologically and Hektoen and Welker [*23*] found that there were at least two antigenic types. Gutman, Moore and Kabat [*24*] simplified the problem to a certain extent by studying serum and urinary proteins from the same subject. But we also oversimplified it a good deal because we had no idea that there were so many proteins present, and we tried to equate the abnormal serum proteins with the urinary proteins.

Recently, a number of investigators have studied the immunochemistry of the myeloma proteins, using all the modern immunochemical technics. To date, the technics which have been used are the quantitative precipitin method developed by Heidelberger and Kendall [*25,26*]; the agar diffusion methods which were developed in France by Oudin [*27*] and in Sweden by Ouchterlony [*28*]; a method of immunoelectrophoresis introduced in the last two years by Grabar and Williams [*29*]; and a procedure involving inhibition of the precipitin reaction, which is a modification of the old Landsteiner

hapten inhibition procedure [30], employed recently by Deutsch et al. [31].

How have people gone about studying these myeloma proteins immunochemically? There

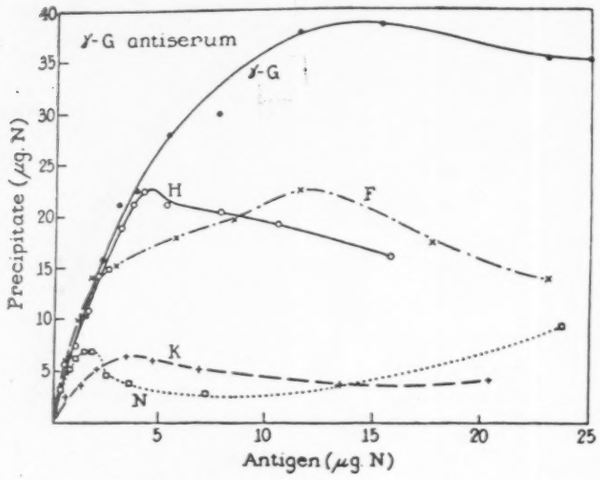

FIG. 11. The quantitative precipitin curves of four myeloma proteins compared with normal γ-globulin using antiserum to normal γ-globulin. (From: SLATER, R. J., WARD, S. M. and KUNKEL, H. G. Immunological relationships among the myeloma proteins. *J. Exper. Med.*, 101: 85–108, 1955 [32].)

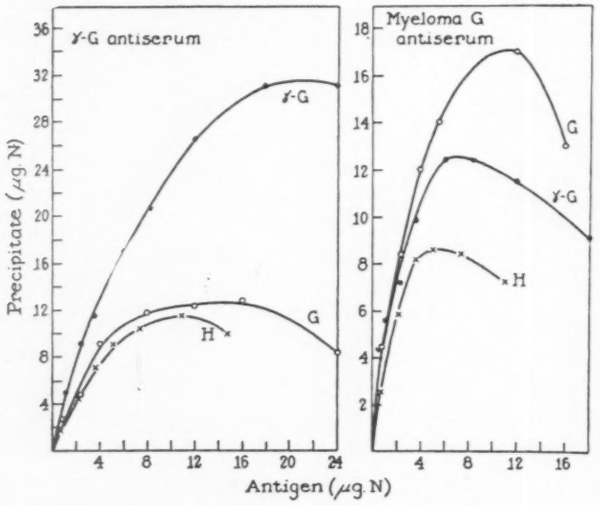

FIG. 12. The quantitative precipitin curves of two myeloma proteins G and H and normal γ-globulin are compared when antiserums to γ-globulin (left) and myeloma G (right) are used. The specificity of each homologous system is apparent. (From: SLATER, R. J., WARD, S. M. and KUNKEL, H. G. Immunological relationships among the myeloma proteins. *J. Exper. Med.*, 101: 85–108, 1955 [32].)

are two obvious ways. Since the myeloma proteins occur in the gamma or other globulin fractions of serum, one may examine purified preparations of normal human gamma globulin. Antiserums are obtained from rabbits immunized with normal gamma globulin, and the reactions of these antiserums with normal and myeloma globulins are studied. Conversely,

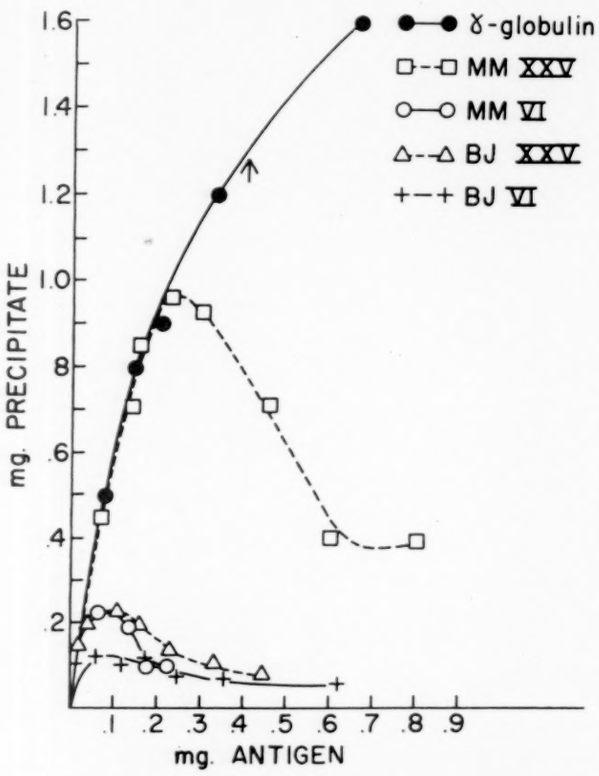

FIG. 13. Quantitative precipitin curves obtained with antigammaglobulin serum (1:4) and normal gamma globulin, myeloma serum globulins (MM XXV and MM VI) and Bence Jones proteins (BJ XXV and BJ VI). The arrow indicates the equivalence zone for normal gamma globulin. (From: KORNGOLD, L. and LIPARI, R. Multiple myeloma proteins. I. Immunological studies. *Cancer*, 9: 183–192, 1956. IDEM. III. The antigenic relationship of Bence Jones proteins to normal gamma globulin and multiple myeloma serum proteins. *Ibid*, 9: 262, 1956 [33].)

antiserums to purified myeloma serum or urinary proteins can be prepared, and the reactions of these antiserums are studied with the homologous antigens, as well as with the normal serum globulin fractions and with other myeloma serum and urine proteins.

With so many people working in this field, it is not surprising that there should be some differences in interpretation of the findings. It is, however, much more important first to consider the large areas in which there is general agreement among investigators. The first point of general agreement is that all abnormal serum and urinary proteins are immunochemically

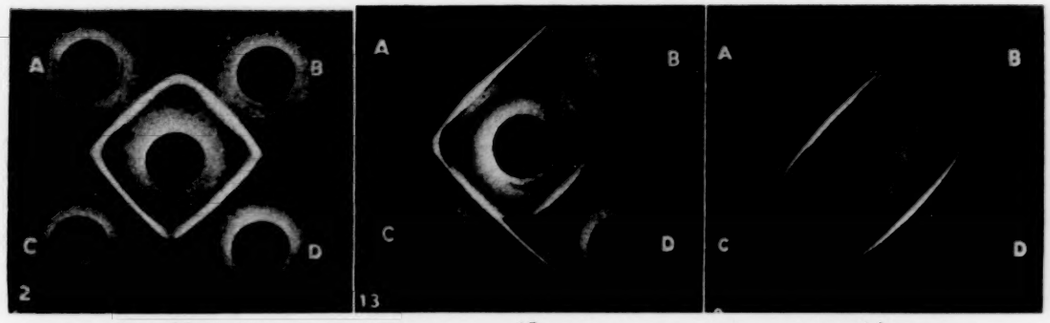

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FIG. 14. Ouchterlony agar diffusion system. Center well contains antiserum to normal gamma globulin. Wells A and B contain the homologous normal gamma globulin antigen. Wells C and D contain the serum globulin from a case of myeloma. (From: KORNGOLD, L. and LIPARI, R. Multiple myeloma proteins. I. Immunological studies. *Cancer*, 9: 183–192, 1956. IDEM. III. The antigenic relationship of Bence Jones proteins to normal gamma globulin and multiple myeloma serum proteins. *Ibid*, 9: 262, 1956 [*33*].)

FIG. 15. Ouchterlony agar diffusion system. Center well: anti-MM IA. Well A, serum MM III. B, Bence Jones protein III. C, Serum MM VIII. D, Bence Jones protein VIII. (From: KORNGOLD, L. and LIPARI, R. Multiple myeloma proteins. I. Immunological studies. *Cancer*, 9: 183–192, 1956. IDEM. III. The antigenic relationship of Bence Jones proteins to normal gamma globulin and multiple myeloma serum proteins. *Ibid*, 9: 262, 1956 [*33*].)

FIG. 16. Ouchterlony agar diffusion system. Center well contains antiserum to normal gamma globulin. A, myeloma globulin MM III. B, Bence Jones protein III. C, Bence Jones protein VIII. D, Myeloma globulin MM VIII. (From: KORNGOLD, L. and LIPARI, R. Multiple myeloma proteins. I. Immunological studies. *Cancer*, 9: 183–192, 1956. IDEM III. The antigenic relationship of Bence Jones proteins to normal gamma globulin and multiple myeloma serum proteins. *Ibid*, 9: 262, 1956 [*33*].)

related to normal gamma globulin, as shown primarily with the quantitative precipitin technic [*25,26*].

In the quantitative precipitin test, known amounts of whatever antigen one wishes to examine are added to a measured volume of antiserum, and the resulting precipitates are centrifuged, washed and analyzed for nitrogen. The amount of precipitate nitrogen is plotted against the amount of antigen nitrogen added. Figure 11 is taken from the work of Slater, Ward and Kunkel [*32*], and shows the typical curve obtained with an antiserum to normal gamma globulin. The two curves in Figure 11 labelled F and H show the results obtained with this antiserum and two different myeloma globulins. These two myeloma globulins happened to have the mobility of gamma globulin. There is apparent cross-reaction between the antiserum to normal gamma globulin and two other myeloma globulins (K and N), which moved faster in electrophoresis, that is of beta mobility. Kunkel and his co-workers believed that the gamma myeloma globulins of patients tended to precipitate more antibody from an antiserum to normal gamma globulin than did the beta myeloma globulins. But, in a more extensive study Korngold and Lipari [*33*] concluded that there was probably no relation to mobility in terms of cross-reactivity.

Figure 12 shows the results obtained with another antiserum to normal gamma globulin, and again it will be seen that the myeloma globulins cross-react and that none of them precipitates all the antibody. The important point is that no instance in which all the antibody to normal gamma has been precipitated by these myeloma globulins has to my knowledge been reported by quantitative technics, although by qualitative precipitin absorption technics there are reports that this may have been accomplished [*34*].

Figure 13 is from some recently published work by Dr. Korngold [33], showing essentially the same thing as the previous figure; an antiserum to normal gamma globulin, a myeloma serum globulin not precipitating all the antibody; another myeloma serum globulin precipitating very little antibody, and two Bence Jones proteins, which were obtained from the urine of a patient, precipitating a small amount of antibody. Other workers have reported similar findings [*34–36*].

We come now to the findings by what is

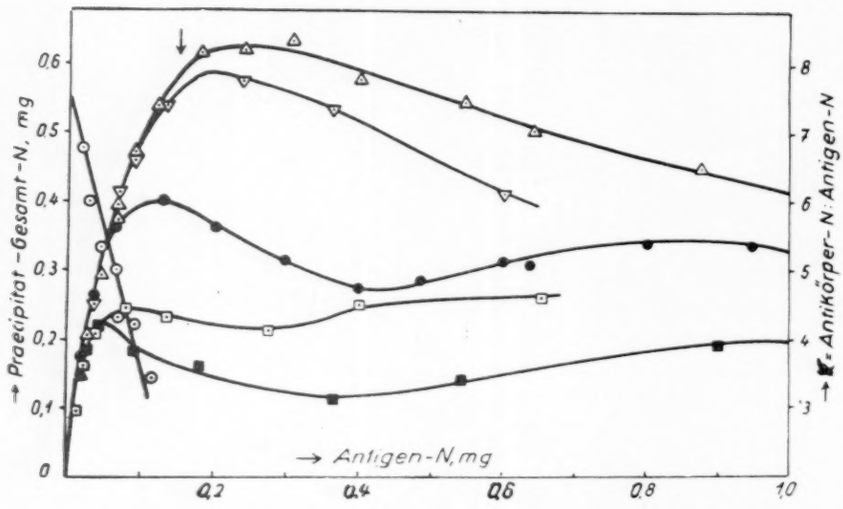

FIG. 17. Quantitative precipitin curves of antiserum against myeloma gamma globulin Z_1 with, Δ homologous antigen myeloma gamma globulin Z_1, ∇ another preparation of this myeloma globulin, Z_2, $\square$ myeloma gamma globulin Li., $\blacksquare$ myeloma gamma globulin S., $\bullet$ normal gamma globulin. (From: (a) LOHSS, F., WEILER, E. and HILLMAN, G. Myelom-plasma-proteine. III. Mitteilung. Zur Immunochemie der γ-Myelom-proteine. *Ztschr. f. Naturforsch.*, 8b: 625–631, 1953 (b) LOHSS, F. and HILLMAN, G. Myelom-plasma-proteine. IV. Mitteilung. Zur Immunochemie der γ- und β-Myelom-proteine. *Ztschr. f. Naturforsch.*, 8b: 706–711, 1953 [*35*].)

called agar diffusion [*27,28*]. This is a rather simple technic. The procedure is somewhat similar to that of making tests for the action of antibiotics on microorganisms. Little wells are made in an agar plate. (Fig. 14.) Into the center well, you place the antibody, and into the others the different antigens to be tested. Wells A and B contain normal gamma globulin. The antigen diffuses toward the antibody well and the antibody diffuses toward the antigen and wherever they meet a precipitate forms. If one is dealing with the same antigen, complete fusion of the two bands occurs at the intermediate point between wells A and B. On the other hand, if one puts a myeloma globulin in wells C and D, they too react with the antiserum and complete fusion of the band occurs between C and D. However, since there is some antibody in the antiserum with which the myeloma globulin does not react, a little spur appears between wells A and C and between B and D. Thus normal gamma globulin contains residual antigenic specificity which is not present in the myeloma globulin. Reactions with other myeloma globulins can be studied, and it has been shown that the myeloma globulins cross-react but are not identical immunochemically with one another or with normal gamma globulin, just as was found by the quantitative precipitin studies [*32,33*].

In Figure 15 an antiserum to a myeloma globulin has been put into the center well, and myeloma serum from two different subjects, III and VIII, has been put in wells A and C. Two diffusion bands are evident. Bence Jones protein from the urine of patients III and VIII is put at B and D and with the urinary proteins only a single band is formed. It is interesting that the serum of this patient had a little Bence Jones protein, because there is a fusion of the urinary protein band with one of the bands obtained with the myeloma serums. Similar findings were also obtained by Burtin et al. [*37*].

Finally, one can see, with an antigamma globulin serum, cross reactions of myeloma serum globulins and urinary Bence Jones proteins. (Fig. 16.) The last three figures were also kindly provided by Dr. L. Korngold [*33*].

Well, so much for the general finding that we get extensive cross reactions with antibody to gamma globulin. Let us turn now and see what happens with antiserums to the abnormal serum proteins. The graph on the right in Figure 12 shows an antiserum to a myeloma gamma globulin, protein G. In this case, the myeloma globulin G precipitates the largest amount of antibody, and normal gamma cross-reacts rather extensively, although not completely. It is of interest that another myeloma globulin H

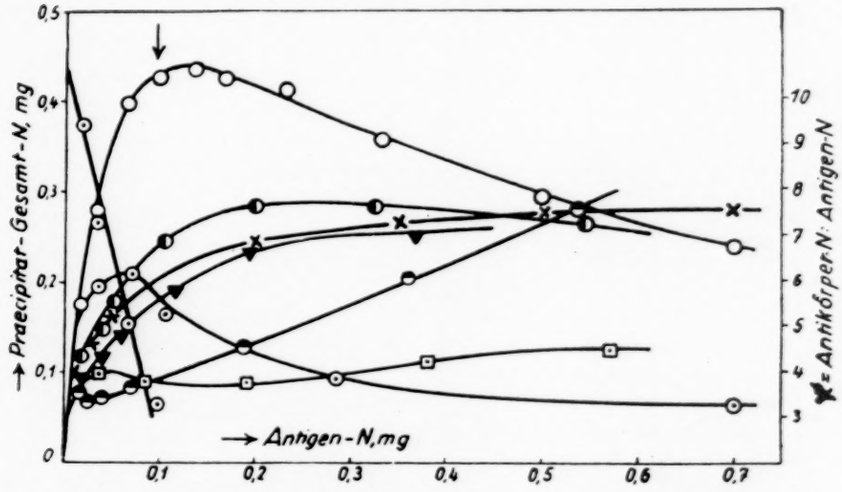

FIG. 18. Quantitative precipitin curves of antiserum (I) against beta-2-myeloma globulin E, and O the homologous antigen, ◓ alpha-2-myeloma globulin L., ◐ beta-2-myeloma globulin B., ▼ beta-1-myeloma globulin Sch., □ gamma myeloma Li., × beta-2-myeloma globulin Sa., ⊙ normal gamma globulin. (From: (a) LOHSS, F., WEILER, E. and HILLMAN, G. Myelom-plasma-proteine. III. Mitteilung. Zur Immunochemie der γ-Myelom-proteine. *Ztschr. f. Naturforsch.*, 8b: 625–631, 1953. (b) LOHSS, F. and HILLMAN, G. Myelom-plasma-proteine. IV. Mitteilung. Zur Immunochemie der γ- und β-Myelom-proteine. *Ztschr. f. Naturforsch.*, 8b: 706–711, 1953 [*35*].)

may not cross-react nearly as extensively as does normal gamma.

Figure 17 is from the work of Lohss [*35*] in Germany. Again it shows an antiserum to a myeloma gamma globulin Z_1. The top curve is the curve with the homologous antigen; a superimposable curve was obtained with another preparation Z_2 from the same patient throughout the antibody excess region. There are some differences in the antigen excess zone, but I do not know whether or not they are too significant. With this antiserum, two other myeloma globulins and normal gamma globulin also cross-react.

Figure 18 shows the variation in amount of specific precipitate obtained with different preparations, with an antiserum to a beta myeloma globulin E [*35*].

In Figure 19 the top curve shows the reaction of another antiserum to beta myeloma globulin (E), with its own antigen. If you take out as much antibody as you can with another myeloma globulin (Li), you leave a fair amount of antibody that can be precipitated by the original myeloma globulin. Similarly, if the antiserum were absorbed with normal gamma, a substantial amount of antibody to the homologous antigen would be left. As a matter of fact, for one of the curves in Figure 18 the antibody was taken out, not with normal gamma but with serum from a subject in the same blood group as the patient, and even whole serum did not remove the antibody to the myeloma proteins completely.

There is still another technic, which has been used by Deutsch [*31*], to show such relationships. (Table II.) With an antiserum prepared in a rabbit by injecting a urinary Bence Jones protein, it was found that on adding 20 μg. of antigen (the Bence Jones protein) to 1 ml. of antiserum, 106 μg. nitrogen were precipitated. However, if 115 μg. of $gamma_2$ globulin N were added to the same amount of antiserum, no precipitate was formed; $gamma_2$ globulin therefore did not precipitate this antibody to Bence Jones protein. But if, to this solution in which he got no precipitation, Deutsch added the same amount of Bence Jones protein which ordinarily would have formed 106 μg. of specific precipitate N, only 55 μg. of precipitate N resulted. In other words, the normal $gamma_2$ globulin had competitively combined with a portion of the antibody to prevent the homologous Bence Jones protein from precipitating it; and in this case it amounts to almost half the antibody. Table II shows that myeloma gamma globulin, $gamma_1$ globulin, normal human serum and another Bence Jones protein gave similar results.

The results with gamma$_1$ globulin and the other Bence Jones protein are complicated since these materials precipitated some of the antibody.

To recapitulate, despite the extensive cross reactions which occur, there is no quantitative demonstration of identity between any myeloma

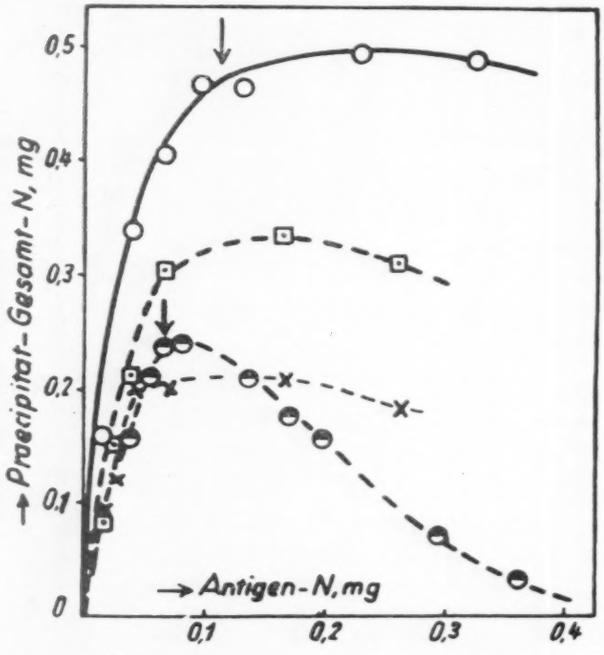

FIG. 19. Quantitative precipitin curves of antiserum (II) against β_2-myeloma globulin E and, ○ homologous antigen before absorbtion, ⊡ homologous antigen after absorbtion with myeloma γ-globulin Li., × homologous antigen after absorbtion with myeloma β_2-globulin Sa., ◓ homologous antigen after absorbtion with whole serum of subject in the same blood group. (From: (a) LOHSS, F., Weiler, E. and HILLMAN, G. Myelom-plasma-proteine. III. Mitteilung. Zur Immunochemie der γ-Myelom-proteine. *Ztschr. f. Naturforsch.*, 8b: 625–631, 1953 (b) LOHSS, F. and HILLMAN, G. Myelom-plasma-proteine. IV. Mitteilung. Zur Immunochemie der γ- und β-Myelom-proteine. *Ztschr. f. Naturforsch.*, 8b: 706–711, 1953 [*35*].)

globulin and normal gamma globulin or between any myeloma globulin and any other myeloma globulin.

On the basis of these data, to which there is no substantial objection except that one has to take for granted that all these investigators were very careful to ensure that when they immunized their animals they got antibody only to a single component and were not dealing with mixtures of antibodies, we now come to the question, what do these data mean? This is, of course, the area in which there are differences of opinion.

As has been said frequently, there are always two possibilities. One of these is, since normal gamma globulin is not homogeneous and has a broad range of mobility, different N terminal amino acids, and the like, and since the myeloma globulins give very sharp peaks, that there are, as Dr. Putnam indicated, in normal sub-

TABLE II*

RESULTS OF BLOCKING REACTIONS OF RABBIT BJ-ZE ANTIBODY REACTION BY VARIOUS PROTEINS

BJ-ZE Added (γ N)	Blocking Protein	Amount (γ N)	Ppt. (γ N)	Ppt. Blocked (γ N)
20.1	.....	0	106	....
0	γ_2-Globulin	115	0	....
20.1	"	115	55	51
0	γ_2-KL	236	0	....
20.1	"	236	75	31
0	γ_1-Globulin	213	18	....
20.1	"	213	58	66
0	Human serum	4880	0	....
20.1	" "	4880	56	40
0	BJ-LP	258	26	....
20.1	"	258	71	61

* (From: DEUTSCH, H. F., CRATCHOVIL, C. H. and REIF, A. E. Immunochemical relation of Bence-Jones protein to normal serum proteins. *J. Biol. Chem.*, 216: 103–111, 1955 [*37*].)

jects small amounts of components identical with the myeloma globulins.

The second possibility is that these myeloma globulins have no normal counterpart in serum and are truly abnormal globulins. It seems a little odd to me that, with so many different proteins as have been found and in view of the difficulty of studying more than one or two of them in great detail, nobody has considered the possibility that some might be normal and present in small amounts, and others might be abnormal. But one can speak only for the proteins which have been thoroughly studied.

To consider these two hypotheses, we have to say something about the immunochemical behavior of normal gamma globulins. We know that there are two kinds of gamma globulin, gamma$_2$ globulin, the predominant component, and gamma$_1$ globulin; as I mentioned, the latter moves more rapidly in electrophoresis, is poly-dispersed in the ultracentrifuge, has components of higher molecular weight, and makes up but a small portion of the total gamma globulin. Most investigators are in agreement that gamma$_2$ globulin represents, by and large, a

single immunochemical entity. Indeed by electrophoresis no distinct peak corresponding to gamma$_1$ globulin can be seen in whole serum and preparations of gamma$_1$ globulin always contain large amounts of gamma$_2$ globulin.

By immunoelectrophoresis (Fig. 20) Grabar and Williams [*29,37*] have found a single peak with gamma globulin preparations. I should like to take a few moments to explain immunoelectrophoresis and its significance. A photographic glass plate is layered with agar. A little hole is cut out in the center of the plate and serum mixed with agar is poured into it and allowed to harden. Then an electric current, with the positive and negative charges as indicated, is passed through the agar. The serum proteins migrate so that the albumin moves the greatest distance toward the anode, and the slow-moving gamma globulin is carried toward the negative pole by electroendosmosis. Then, another piece of the agar is cut out to form a trough parallel to the direction of migration of the protein components and a mixture of the antibody in agar is poured onto the plate and allowed to harden. The various protein components previously spread out by the electric current and the antibody diffuse toward each other. Wherever they meet, there is a band of precipitation. The interaction of the electrophoretically spread out components of serum with the various antibodies gives rise to the set of bands shown in the lowest portion of Figure 20 [*37*]. Although this antiserum was prepared to whole normal human serum and contained antibody to many antigens, you will notice nearest to the pole a very sharp band of broad mobility, but nevertheless a single band, due to gamma globulin. With the same antiserum, the upper sections show that several purified normal gamma globulin fractions gave only a single band by this technic, providing evidence that normal gamma globulin consists only of a single antigenic component [*29,37,38*].

The next piece of evidence may be derived from quantitative precipitin data. (Fig. 21.) With a pool of rabbit antiserum to normal gamma$_2$ globulin, several preparations of gamma$_2$ globulin, made in different laboratories, were compared in quantitative precipitin curves [*39*]. You notice (Fig. 21) that they give a single quantitative precipitin curve up to the point of maximum precipitation. All gamma$_2$ globulin preparations which we have studied have given similar results. Other investigators have obtained

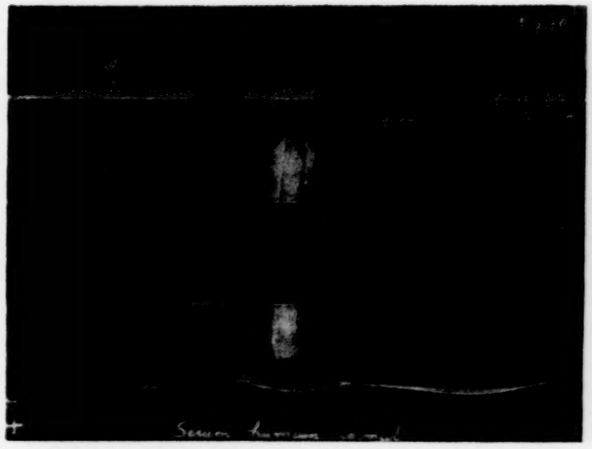

FIG. 20. Electrophoresis in agar of three different subfractions of human gamma globulin and of normal human serum (see text). Precipitin reactions were obtained with a horse antiserum prepared to normal human serum. (From: GRABAR, P. Que sont les gamma-globulenes? *Therapie*, 9: 163–180, 1954 [*37*].)

similar data (Jager [*16*], Cohn et al. [*40*]). On the other hand, a preparation of gamma$_1$ globulin, of faster mobility, gives another curve, which reaches the same maximum if enough antigen is added, and requires about 50 per cent more material by weight to precipitate an equal amount of antibody. If one reads off from the curves (Fig. 21) the quantities of gamma$_2$ and gamma$_1$ globulins to give the same quantity of specific precipitate N, the ratio is about 2 to 3. We would say, therefore, that under these circumstances the fast-moving gamma$_1$ material reacts with antibody as if it contained about two-thirds by weight of an antigen exactly like the gamma$_2$ globulin.

Some investigators [*40*] have thought that there are minor differences among gamma$_2$ globulins attributable to antigenic impurities and that these can be detected by studies in the inhibition zone. However, as Grabar [*41*] predicted, Williams [*42*] has reported that these findings may also be attributable to differences in the solubility of the specific precipitates formed with various gamma globulins and have antibody, depending upon the proportions of eu- and pseudo-globulins present in the gamma$_1$ and gamma$_2$ preparations.

Now, what about these hypotheses? For hypothesis 1 to be correct, (that all myeloma globulins are present in small amounts in normal serum) the immunochemical behavior in quantitative precipitin studies of the antigamma$_2$ globulin with normal gamma$_2$ globulin would have

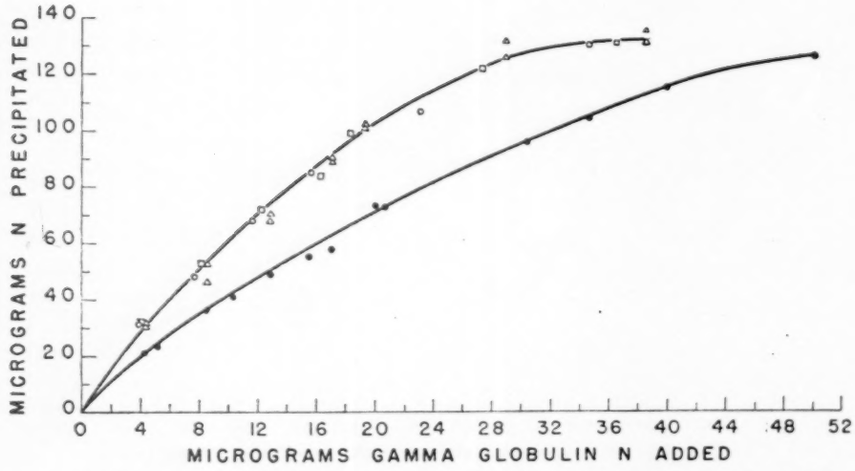

FIG. 21. Reaction of γ_2 and γ_1-globulins with rabbit anti-γ-globulin Pool XI. Δ = Sample B; $\square$ = fraction II-1,2; $\circ$ = fraction II-3; $\bullet$ = γ_1-globulin. (From: KABAT, E. A. and MURRAY, J. P. A comparison of human γ-globulins in the reactivity with rabbit anti-γ-globulin by the quantitative precipitin method. *J. Biol. Chem.*, 182: 251–259, 1950 [*39*].)

to be a composite curve, the resultant of a large number of precipitin curves, each produced by different globulins of different immunochemical reactivity, each giving a different amount of specific precipitate at its maximum. Were this the case we should not expect to find the superimposable curves (Fig. 21) with different preparations of normal gamma$_2$ globulin fractionated in various ways.

Also, for hypothesis 1 to be correct one would ultimately have to find that all the antibody to a myeloma serum protein was precipitated by a normal gamma globulin. Slater, Ward and Kunkel [*32*] isolated a number of fractions of different mobility from whole normal serum by electrophoresis on starch but none of them could ever remove all the antibody to the myeloma globulin (Fig. 22); so about all one would be inclined to say is that there is no evidence as yet to show that these proteins are normal globulins, present in small amount.

However, the inhibition experiments of Deutsch et al. [*31*] (Table II) suggest the possibility that even the electrophoretically separated normal gamma globulin fractions may contain several components, one of which might, if it were present alone, precipitate all the antibody to the myeloma globulin. However, precipitation could be inhibited because of the presence of the other normal gamma globulin constituents. Thus we still do not have a definite and conclusive answer to permit one to choose between the two possibilities.

I should like to take one minute, in concluding, to touch on the question of what we are looking at when we see these different immunochemical reactivities. Most people have tended to think that perhaps immunochemistry provides information about the entire structure of the protein molecule, but this is definitely not so.

From studies which we have been carrying out with a simple antigen, dextran, a polymer composed of a single sugar–glucose, we have obtained data indicating that the combining site on an antidextran molecule has a structure which has dimensions complementary to an area about as big as from 4 to 6 glucose rings [*43*]. This is a small structure and represents the total complementary structure on the antidextran molecule to a single antigen grouping.

Therefore, if we have, as Dr. Putnam has shown you, 1,300 amino acids making up a globulin molecule, and even if we have five or six antigenic sites involving dimensions of the size of 4 to 6 glucose rings, the immunochemical method merely picks up a number of small bumps on an extremely large molecule. With respect, therefore, to the whole question of the origin of these globulins, it should be remembered that we are still studying only a small portion of the total molecule by immunochemical methods, and therefore cannot say anything about the rest of the molecule, whether it is normal or abnormal.

DR. CALVIN PLIMPTON: If I understood him correctly, Dr. Osserman implied that virtually

all cases of primary amyloidosis are probably atypical forms of multiple myeloma. Yet at pathologic conferences we have presented instances of primary amyloidosis in which a careful autopsy has revealed no signs of proliferation of either plasma cells or lymphocytes. I wonder if Dr. Osserman would comment on this apparent contradiction.

DR. OSSERMAN: I should like to thank Dr. Plimpton for phrasing his question with the reservation of "virtually all." Within the past year we have had occasion to observe two subjects who displayed the characteristic clinical and pathologic features of so-called primary amyloidosis, in whom repeated marrow aspirations and subsequent postmortem bone examinations failed to demonstrate significant increases in plasma or myeloma cells. The marrow of one of these patients displayed hypoplasia of all elements of the erythroid, myeloid and lymphoid series. The second patient showed a maximum of 6 per cent plasma cells in several sites examined.

It should be stressed that these two cases were the exceptions. There are at least three possible explanations. We are all aware of the fact that marrow aspirations, and indeed autopsy studies of bone sections, are sampling procedures of a very large and, at times, a very pleomorphic organ, *viz.*, the bone marrow, Likewise, the postmortem examination, as routinely performed, provides only a small sector of this organ for study. Certainly it is conceivable that a limited area of pathologic proliferation of abnormal cells may be missed. The second possibility which was suggested by one of the cases is that on occasion this process may proceed ultimately to a "burnt-out," hypoplastic stage. To date, this progression has not been documented. A third possibility, and one which is deserving of extensive probing, is the obvious one, that is, that primary amyloidosis is a clinicopathologic syndrome which may have more than one etiology. This possibility cannot be denied, but again it is noteworthy that a plasma cell excess can be demonstrated in the great majority of these cases.

STUDENT: Do you need this complicated electrophoresis apparatus to pick up these abnormal proteins, or is there a simple way in which they can be identified?

DR. OSSERMAN: The technic of filter paper electrophoresis is a simple procedure. A practical unit which will satisfy the vast majority of clinical needs can be assembled for well under one hundred dollars and operated by a technician trained in routine chemistry. More elaborate and more costly units are available, but for most purposes of clinical practice a simple and inexpensive unit will prove adequate.

DR. EDWARD E. FISCHEL: I think it is only fair to say that the lymphocyte and the plasma cell

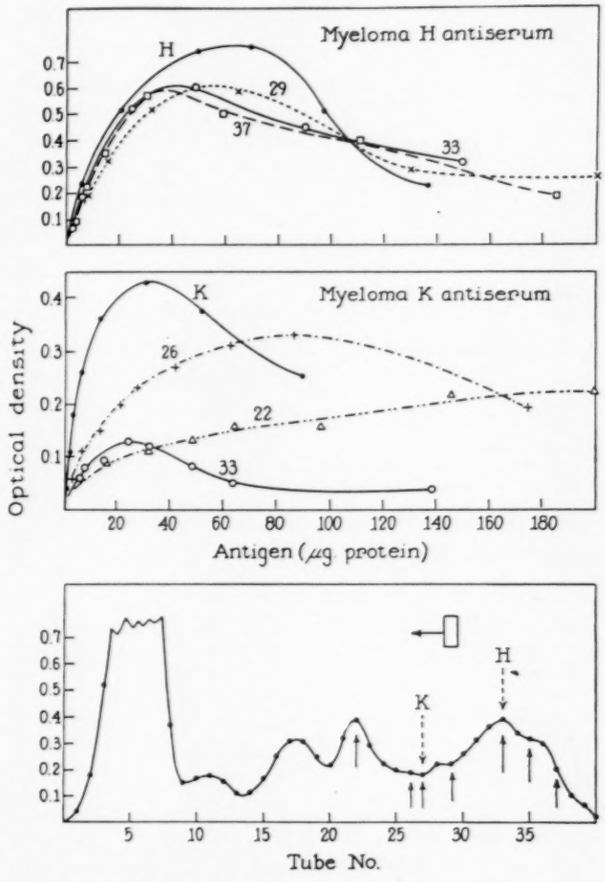

FIG. 22. The lowest pattern illustrates the zone electrophoretic separation of normal serum. Origin and direction of migration indicated by box and arrow. Broken arrows indicate mobility of two of the myeloma proteins whose antiserums were tested. Solid arrows indicate fractions of normal serum used for testing. The upper two patterns demonstrate curves of quantitative precipitin reactions between numbered fractions and the myeloma antiserums. (From: SLATER, R. J., WARD, S. M. and KUNKEL, H. G. Immunological relationships among the myeloma proteins. *J. Exper. Med.*, 101: 85–108, 1955 [*32*].)

are not the only cells that produce gamma globulin. In tissue culture experiments, years ago, Landsteiner and Parker had rabbit fibroblasts growing in chick plasma and, after successive generations, they continued to elaborate a protein which was identifiable immuno-

logically as a rabbit serum globulin of gamma mobility.

DR. OSSERMAN: Dr. Fischel's point is well taken. Comparable tissue culture experiments with rabbit plasma cells have also indicated gamma globulin formation *in vitro*. We have perhaps given too great emphasis to the roles of the plasma cell and lymphocyte in globulin synthesis. We should also stress that there may well be a vast number of other serum globulins which have an electrophoretic mobility in the range of gamma globulin, and, among these, there may be globulins which are of fibroblastic or other cell origin. It is noteworthy, however, that among the neoplasias it is only those of plasma cell and lymphocytic origin which display these remarkable protein excesses.

SUMMARY

DR. RICHARD J. CROSS: For many decades multiple myeloma has been diagnosed on the basis of certain clinical and pathologic manifestations. In almost all these patients an abnormal, electrophoretically homogeneous protein can be found in either the plasma or urine or both. Similar proteins can be found in a few patients with other diseases, specifically lymphomas on the one hand and primary amyloidosis on the other, suggesting that certain instances of these conditions may actually represent atypical forms of multiple myeloma.

Each patient with myeloma would appear to produce his own individual protein, different from that formed by any other patient. And the characteristics of this protein remain unchanged throughout the course of the disease. It is tempting to speculate that normal subjects have thousands of plasma proteins, each produced by a different group of plasma cells. If one cell undergoes malignant change, its particular protein is then overproduced at the expense of the other globulins. But no proof of such an hypothesis has as yet been forthcoming. There is immunochemical evidence which suggests that these myeloma proteins may be truly abnormal and not present in healthy subjects even in minute amounts.

The urinary proteins are distinct and different from those found in the plasma, and most, but not all, will yield a positive Bence Jones test. As in the case of plasma proteins, each patient appears to produce his own unique urinary protein, which is homogeneous on electrophoresis and in the ultracentrifuge, and which differs from the protein of any other patient in certain physical and/or chemical characteristics.

Isotope studies of the rates of formation and turnover of these proteins indicate that Bence Jones protein is rapidly formed and is promptly excreted in the urine. The abnormal plasma globulin, on the other hand, contains far less isotope and is turned over much more slowly than is the urinary protein. Thus it would appear clear that the Bence Jones protein is not derived from the atypical globulin but is formed *de novo* from the amino acids of the body.

The fact that approximately 97 per cent of patients with myeloma have an abnormal protein in the plasma and/or urine means that the identification of these substances can be of great assistance in the diagnosis of this often confusing disease. Fortunately, quite simple, commercially available equipment for the paper electrophoresis of proteins makes such identification possible in many laboratories.

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Clinico-pathologic Conference

Tender Pelvic Mass, Fever, Jaundice and Melena

STENOGRAPHIC reports edited by Lillian Recant, M.D. and W. Stanley Hartroft, M.D. of weekly clinico-pathologic conferences held in the Barnes and Wohl Hospitals are, published in each issue of the Journal. These conferences are participated in jointly by members of the Departments of Internal Medicine, Preventive Medicine and Pathology of the Washington University School of Medicine and by Junior and Senior medical students. In the present conference an unusual departure from protocol was occasioned by the visit of Dr. Hans Popper of The Mt. Sinai Hospital in New York. We should like to express our appreciation to him for conducting and editing the pathologic portion of this conference.

THIS twenty-four year old "26" girl (P. M. 793-51) was admitted to Cook County Hospital on June 26, 1951. She stated she had been reasonably well until November, 1950, when she had had lower abdominal pain for which she was treated with injections for several weeks. She was told that she had pelvic inflammation. In February, 1951, she had a cough, chilly sensations, fever and pain in the chest for which she was given penicillin by her doctor, who diagnosed pneumonia. For the four weeks prior to the present admission she had been bothered by a recurrence of this lower abdominal pain which seemed to spread toward the epigastrium and then to the left side and flank. In addition, there had been some vaginal discharge. During this time her appetite had become poor and she thought she had lost weight. Occasional nausea and vomiting had also occurred, and during the last week before admission the patient stated that she moved her bowels only once and that was with the aid of several enemas. The week prior to admission she stated her urine became dark and her stools light in color. Shortly thereafter she noticed a yellow tinge to her eyes.

System review revealed no significant cardiovascular, respiratory or urinary symptomatology. Past history was non-contributory. The patient admitted to a fairly heavy alcohol intake (12 ounces/day). She denied the use of drugs.

Physical examination revealed the patient to be a well developed, well nourished, white female, who appeared to be in moderate distress from abdominal pain which was aggravated by movement. Temperature was 98°F.; pulse, 80; respirations, 18; blood pressure, 120/70 mm. Hg. There was moderate scleral and skin icterus but no significant enlargement of the lymph nodes and no spider angiomas were noted. Examination of the head and neck was within normal limits. The chest was symmetric with equal excursions. Rhonchi and wheezes without rales were scattered throughout her chest. Resonance was unimpaired and both leaves of the diaphragm moved freely. The heart was normal. The abdomen showed rather marked tenderness over the liver and in both lower quadrants. A walnut sized, slightly tender mass was felt just above the middle of the right inguinal ligament. There was marked tenderness in the left flank. An indistinct mass could be felt in this area when the patient was placed in the right lateral decubitus position. It was the examiner's impression that this mass was spleen. Pelvic examination caused considerable pain and could not be adequately performed; however, the impression was that there was a tender, bulging adnexal mass present on the right side. Rectal examination revealed a fecal impaction of brown stool which was benzidine negative. Congenital deformities of the thumb and first finger were noted.

Laboratory studies revealed the hemoglobin to be 14.1 gm. per cent; red blood cells, 5.27 million per cu. mm. and white blood cells, 11,900 per cu. mm. with a differential count of 67 per cent polymorphonuclears, 11 per cent band forms, 1 per cent eosinophils, 17 per cent lymphocytes and 4 per cent monocytes. The urinalysis on admission was negative except for

TABLE 1
SUMMARY LABORATORY DATA

Tests	6/27	7/1	7/5	7/9	7/16	7/24	7/31	8/7	8/13	8/20	8/21	8/21	8/24
Cephalin flocculation	0	....	0	0	2+	2+	0	0	....	....	1+	....	....
Thymol turbidity (units)	17.8	....	8.9	11.2	13.0	18.4	18.2	5	....	....	6.3	....	....
Total cholesterol (mg. %)	596	750	725	1000	910	960	1048	972	....	604	724	....	....
Esters/total (%)	....	....	47.5%	....	....	....	....	43.2%	....	....	....	....	....
Lipid phosphorus (mg. %)	....	....	54.4	....	....	....	....	125	....	....	....	....	....
Alkaline phosphatase (units)	21.7	33.7	28.1	40.0	64.3	285	23	39.8	....	....	13	....	....
Total bilirubin (mg. %)	....	....	28	....	....	....	....	22	....	....	....	....	....
Icterus index	110	172	.....	201	182	119	182	104	....	81	86	....	....
Howe albumin (gm. %)	....	4.8	5.4	4.0	3.3	4.0	....	3.38	....	....	....	....	....
Howe globulin (gm. %)	....	2.2	2.6	2.6	3.1	2.2	....	2.12	....	....	....	....	....
NPN (mg. %)	32	....	.....	32	....	26	....	30	40	....	30	....	....
Cl (mEq./L.)	....	....	.....	....	....	....	....	.....	104	85	....	91	102
Na (mEq./L.)	....	....	.....	....	....	....	....	.....	134	117	....	121	125
CO_2 (vol.%)	....	....	.....	....	....	....	....	.....	40	42	....	50	32
K (mg. %)	....	....	.....	....	....	....	....	.....	....	....	13.3	12.5	23.4
P (mg. %)	4.3	4.9	2.9	4.4	4.7	5.3	4.6	3.4	....	....	....	....	....

the presence of bile. No urobilinogen was found. Serologic reaction was negative. Cervical smears and stool cultures revealed no pathogens. An electrocardiogram showed only sinus tachycardia. The liver function studies which were obtained on admission and throughout hospitalization are recorded in Table 1. Roentgenograms taken on admission revealed the chest and abdomen to be within normal limits.

The course in the hospital was characterized by a low grade fever and by the persistence of jaundice, abdominal pain and the presence of a mass in the right lower quadrant. At no time was urobilinogen found in the urine. On July 3, 1951 roentgenographic examination of the gastrointestinal tract revealed the esophagus, stomach, duodenum, rectum and colon to be normal; however, a concave indentation on the medial aspect of the cecum was noted and was presumed to be due to extrinsic pressure. The patient's condition during this time was little altered, save for the development of diarrhea described as containing mucus and bright red blood. On July 8, 1951, two weeks after admission, examination revealed the liver to be palpable 5 cm. below the costal margin. There was no evident splenic enlargement. Marked lower abdominal tenderness with guarding was noted with no significant rigidity. Vaginal examination revealed a normal cervix which appeared to be bound down to an exquisitely tender orange-sized posterior mass, which bulged backward and to the right. Rectal glove revealed light brown feces stained with red blood. A gynecologic consultant thought the patient had an inflammatory mass in the cul-de-sac.

At this point, diagnostic impressions were strongly divided. Exploration was advocated by one group, and considered contraindicated by another. Liver biopsy was recommended, but the prothrombin time was 15 per cent. The patient was treated with vitamin K, and after two weeks the prothrombin time rose to 55 per cent. On August 8, 1951 a liver biopsy was performed. Histologic changes were reported to be compatible with posthepatic obstruction and exploration was recommended. However, there was marked pain and tenderness, and a hematoma developen at the site of the liver biopsy. At this time the hemoglobin level dropped to 3.8 gm. per 100 cc. and the hematocrit was 17 per cent. A leukocytosis of 19,800 was also present. Sedation and blood transfusions were given, although the blood pressure never fell precipitously. Subsequently increasing abdominal distension developed, and it was thought that a hemoperitoneum was present, giving rise to a paralytic ileus. Treatment consisted of suction and the administration of fluids intravenously. By August 10, 1951, two days after the biopsy, the patient's bowels began to move and she appeared much as she had prior to the biopsy. The stools were now described as tarry black. The abdomen remained distended.

On August 11, 1951, further radiologic studies were made. The examination of the chest showed

an opacity in the left costophrenic sinus and an area of ill-defined density in the right costophrenic angle. Roentgenogram of the abdomen showed scattered gas-filled loops of bowel in no diagnostic pattern. A repeat examination of the chest on August 16, 1951 confirmed the findings previously reported.

By August 20, 1951 the patient was in marked electrolyte imbalance. (Table 1.) Intensive electrolyte therapy was instituted. She had continuous diarrhea, which was described as "watery, pasty white." By August 25, 1951 she was semicomatose. Her hemoglobin was 10 gm. per cent and the white blood count was 20,400 per cu. mm. She died on August 26, 1951, the sixtieth hospital day.

CLINICAL DISCUSSION

DR. EDWARD REINHARD: The first point we must clear up before discussing this case is the possible epidemiology of this patient's disease and the nature of her occupational hazards. Dr. Shank, can you tell us what a "26" girl is?

DR. ROBERT SHANK: I have been told that a "26" girl is a dice handler at a gambling table. What else she may do, I have not been able to ascertain.

DR. REINHARD: Dr. Allen, two weeks after this patient's admission to the hospital, a gynecologic consultant thought she had an inflammatory mass in the cul-de-sac. Would you give us your ideas concerning the nature of the inflammatory pelvic process and its possible complications. Might it have been associated in any way with the later development of jaundice and liver disease?

DR. WILLARD ALLEN: The patient's illness probably began in the pelvic organs some months before her admission to the hospital with jaundice. The nature of this illness was obscure. Although it is reported that she had pelvic inflammatory disease, we cannot assume that the etiology was specific. She could well have had a tubular pregnancy, or any number of other troubles. When we are faced with a mass—and I would judge this was a fairly substantial tender mass, palpable both vaginally and rectally—we assume it may either be an inflammatory mass or a tumor. If it were a tumor, it would have to be a malignant tumor in order to explain the whole story. On the other hand, most patients with malignant pelvic tumors do not have fever, even though fever may be present with extensive carcinomatosis. If this patient had an inflammatory mass, then it would be quite unusual for the ordinary pelvic infection to proceed in this general manner. By ordinary pelvic infection, I mean gonorrheal infection with or without secondary invading organisms. There are, of course, other types of pelvic infection which could cause a prolonged fever, for instance tuberculosis or actinomycosis of the pelvic organs. We have seen three patients with actinomycosis on our service, one of whom died; the other two recovered following surgery. None of these three patients had jaundice. Frankly, it is very unusual to see jaundice in a patient with primary pelvic pathology. There are some ovarian tumors of the pseudomyxomatous variety, which spread through the peritoneal cavity and occasionally produce symptoms in the upper abdomen. I have seen two patients with pseudomyxoma peritoneum who had portal obstruction but not biliary obstruction.

DR. REINHARD: Would the age of this patient, twenty-four years, make tumor unlikely?

DR. ALLEN: No, the age is of no consequence in the consideration of malignancy of the ovary.

DR. REINHARD: One of the major problems, Dr. Shank, is to define the type of jaundice that this patient had. Her urine consistently contained bilirubin but no urobilinogen.

DR. SHANK: The laboratory data recorded are typical of biliary obstruction in every regard and with little if any evidence of parenchymal hepatic damage.

DR. REINHARD: Would you say that the data are indicative of extrahepatic obstruction of the biliary tract or could the obstruction be intrahepatic?

DR. SHANK: It could be either intra- or extrahepatic obstruction. The evidence also indicates that obstruction was total in terms of the elevation in serum cholesterol and in phospholipid, and in the absence of urine urobilinogen.

DR. REINHARD: Dr. Moore, do you have any comment to make upon the intrahepatic or extrahepatic nature of this obstruction?

DR. CARL MOORE: I agree entirely with what Dr. Shank has said about the interpretation of the data. The protocol states, however, that that stool was brown in color. If the stool was really brown, the obstruction was incomplete and we have to assume that the urobilinogen determinations in the urine may not have been correct.

DR. REINHARD: Dr. Karl, what do you consider the etiology of the liver disease and of the obstructive jaundice?

DR. MICHAEL KARL: From the standpoint of primary disease, cholangiolitic hepatitis could give us all the laboratory findings and would be consistent with the clinical course. In considering extrahepatic obstruction, one might consider the possibility of obstructing nodes in the portahepatis originating from the underlying pelvic pathology. A third possibility that should be mentioned is portal thrombosis, either septic or otherwise, again associated with the basic pelvic disease.

DR. REINHARD: Which of the possibilities do you think is the most likely?

DR. KARL: Cholangiolitic hepatitis, although this would imply making separate diagnoses in the liver and in the pelvis.

DR. REINHARD: Dr. Shank, do you agree?

DR. SHANK: It is tempting to try to tie all her troubles together. Cases of pylephlebitis have been described with perihepatic abscess evolving from sources of infection within the pelvis as well as from about the appendix. This would be a rare event, however. Moreover, occasionally infection may spread by lymphatics and may involve lymph nodes, producing abscesses about the portahepatis. This also would be a rare event. My opinion is conditioned somewhat by Dr. Popper's appearance here today. I, therefore, think the process is very likely cholangiolitic hepatitis, occurring in a patient who also has pelvic inflammation.

DR. REINHARD: Dr. Karl, a needle biopsy of the liver was carried out, following which hemoperitoneum and paralytic ileus developed. I recall one patient who died with massive hemoperitoneum shortly after a liver biopsy was performed. She turned out to have miliary tuberculosis with extensive tuberculosis of the liver. Is hemoperitoneum following liver biopsy more common in patients who have inflammatory liver disease as opposed to other types of liver disease?

DR. KARL: There are two situations in which hemoperitoneum more commonly occurs. One is diffuse liver disease with so much widespread damage that the prothrombin concentration is low enough to be associated with bleeding. In addition, bleeding may also be seen in patients with widespread metastatic disease of the liver.

DR. REINHARD: Following the needle biopsy of the liver, roentgenograms of the chest were taken. The diaphragms were elevated and there was discoid atelectasis of both lower lobes. There was also a small pleural effusion on the right although this finding was not specifically mentioned in the official interpretation. Dr. Sherry, the elevated diaphragm and the atelectasis can be attributed to the hemoperitoneum and resulting paralytic ileus. Would you comment on the significance of right pleural effusion under these circumstances?

DR. SOL SHERRY: The presence of pleural effusion in association with hepatic disease is not an unusual event. Certainly one may find fluid in the chest in the presence of a liver abscess. The usual explanation for pleural effusions associated with ascites is that ascitic fluid traverses the diaphragm through lymphatic or other vascular channels. Did you want me to comment on any other aspect of this case?

DR. REINHARD: Yes, as you wish.

DR. SHERRY: The presence of Dr. Popper should not sway us in any particular direction since he has written extensively about various types of liver disease. It may be noted, however, that Dr. Popper wrote an article in 1937 [*1*] which indicated that there was a significant incidence of jaundice in patients with gonorrhea. Subsequently, others suggested that most patients observed had received a variety of injections and may have been suffering from hepatitis. Perhaps we are dealing here with a cholangiolitic type of hepatitis in association with the therapy of gonorrhea. On the other hand, in 1930 Dr. Curtis described the appearance of "violin string" adhesions in the portal bed area as well as on the anterior surface of the liver in association with gonorrheal inflammatory disease in the pelvis [*2*]. Subsequently Fitz-Hugh described a number of patients with an acute perihepatitis in association with gonorrhea [*3*]. I wonder whether or not this patient might have had perihepatitis subsequent to pelvic inflammatory disease, which resulted in scarring and obstruction in the portal bed. The pleural effusion and pulmonary symptoms would then represent an episode of perihepatitis rather than pneumonia.

DR. REINHARD: This patient had a reddish stained stool which gave a positive reaction to the benzidine test prior to the needle biopsy of the liver. Three days after the biopsy she began

[1] POPPER, H. and WIEDMANN, A. Über gonotoxischen Ikterus. *Ztschr. klin. Med.*, 131: 258, 1937.

[2] CURTIS, A. H. A cause of adhesions in the right upper quadrant. *J. A. M. A.*, 94: 1221, 1930.

[3] FITZ-HUGH, T., Jr. Acute gonococcic peritonitis of the right upper quadrant in women. *J. A. M. A.*, 102: 2094, 1934.

to pass tarry stools. Dr. Kenamore, to what would you attribute the blood in her gastrointestinal tract?

DR. BRUCE KENAMORE:* It should be noted that before the patient had a pelvic examination her stool was benzidine negative. Apparently there was some difficulty in examining her at first, and I judge she had repeated pelvic examinations. The appearance of bright red blood on the rectal glove after repeated pelvic and rectal examinations could have been due to trauma. The appearance of tarry stools following needle biopsy, however, suggests that either a portion of the gastrointestinal tract was punctured or intrinsic disease of the bowel was present.

DR. REINHARD: Would a tuberculous process which involved the peritoneum, the ileum, cecum or other part of the lower gastrointestinal tract seem plausible as a cause for the bleeding?

DR. KENAMORE: Serosal involvement of the alimentary tract secondary to tuberculous peritonitis does occur. One would have to postulate primary gastrointestinal tuberculosis otherwise to explain the bleeding.

DR. REINHARD: It is interesting that at Seaview Hospital, Stemmerman found tuberculosis of the bile ducts in 3 per cent of 1,500 autopsies on tuberculous patients. These of course were patients in a tuberculosis sanitorium. We cannot dismiss the possibility of a tuberculous infection as the cause of the pelvic disease and of the other manifestations of disease.

DR. CARL HARFORD: In spite of the fact that this lady was a "26" girl, it seems possible that the cause of her abdominal pain was appendicitis and that the abscess in the pelvis was a postappendiceal abscess. This explanation would fit very well with portal vein thrombosis and secondary abscess of the liver.

DR. REINHARD: There seem to be three possible explanations for this patient's death. One is diffuse neoplastic disease involving the pelvis and liver bed. To me this seems unlikely. The other possibilities are that the patient had either pelvic inflammatory disease and cholangiolitic hepatitis, or that she had a primary disease of an infectious nature in the pelvis, possibly tuberculosis, and that the other manifestations of the disease were complications of the spread of this infection. Dr. Hartroft will introduce our guest pathologist.

PATHOLOGIC DISCUSSION

DR. HANS POPPER: The basic problem at the time of admittance to Cook County Hospital was that of a jaundice, obstructive in nature, with a mass in the lower abdomen. The liver biopsy findings indicated moderate cholestasis. The lobular architecture was well preserved; the centrolobular zone revealed considerable bile stasis with plugs in the bile canaliculi. The topical effect of bile upon the liver cells was reflected in an occasional rarification and pigmentation of the cytoplasm designated as feathery degeneration. (Fig. 1, top.) Liver cell damage was not conspicuous. The portal tracts were larger than normal, the connective tissue was arranged in a circular fashion, and moderate increase in the bile ducts but little proliferation of the ductules was apparent. (Fig. 1, bottom.) These changes reflect the hydromechanic effects of an extrahepatic biliary obstruction upon both portal tracts and parenchyma. This picture is in contrast to that of intrahepatic cholestasis of the character of cholangiolitis which only involves the parenchyma. However, in the earlier stages of bile stasis, the differentiation between intra- and extrahepatic cholestasis in biopsy specimens is notoriously difficult and as a rule we register at best an impression, as in this instance. We prefer to commit ourselves only when, in late stages of extrahepatic biliary obstruction, the hydrohepatosis produces bile infarcts in the periportal zone of the parenchyma and bile extravasates in the portal tract itself.

At autopsy the body was deeply icteric; despite the hypercholesteremia no skin xanthomas were noted. The thyroid exhibited both involution and hyperplasia and does therefore not explain the biochemical changes. The lungs showed only some congestion and slight atelectasis in the dependent portions. The heart was small and the intima of the aorta free of significant arteriosclerosis. Apparently arteriosclerosis is not necessarily present in cholesteremia associated with hepatic conditions. The adrenals were depleted of lipids as we would expect in a patient with the history we have here. The kidneys were enlarged, edematous and, especially in the medullary portion, distinctly green pigmented. The diagnosis of biliary nephrosis was confirmed by the microscopic analysis in that the glomeruli were normal while the dilated distal convoluted tubules contained brown pigmented casts, their epithelium being intact. This condition results in a severe jaundice but not in dysfunction of the kidney, similarly as jaundice of the skin does not interfere with its function. Only if in addition an acute

* Died March, 1957.

nephrosis of the character of the lower nephron nephrosis develops as a result of circulatory disturbances, do manifestations of renal failure appear. The spleen weighed 120 gm. and contained foam cells with vacuolated cytoplasm but there was no evidence of portal hypertension as would be found in a cirrhosis.

The liver weighed 1,800 gm. Over the otherwise smooth surface a recent yellow fibrinopurulent exudate was noted. On the cut surface the parenchyma was deep green and the portal tracts distinctly yellow. The lobular architecture was preserved. Microscopically, the stellate shaped portal tracts exhibited extensive fibrosis especially around the bile ducts as well as an increase of the connective tissue as septa along the lobular periphery. This increase resulted in a beginning separation of the lobules in the form of a perilobular fibrosis. The centrolobular zone revealed even more bile stasis than was seen in the biopsy specimen. Hepatocellular damage was still not severe and fat could not be demonstrated. In the portal tract three findings pointed to an extrahepatic biliary obstruction: (1) distinct proliferation of the septal bile ducts without marked reaction of the ductules, (2) large bile plugs or microcalculi in dilated septal bile ducts, indicating the presence of bile stasis not only in the parenchyma but also in the portal tracts, and (3) escape of bile from the bile ducts and bile ductules. (Figs. 2A and B.) These features were also associated with patchy necrosis of liver cells in the periportal zone of the parenchyma, possibly as a result of interference with blood flow because of compression of blood vessels by the accompanying dilated bile ducts. These changes are an expression of a hydromechanically induced hydrohepatosis which is obviously also responsible for the portal fibrosis. All those lesions, considerably aggravated in comparison to the biopsy, force us to the diagnosis of an extrahepatic biliary obstruction. However, both the common and the main hepatic duct were narrow, and calculi, tumors or strictures were not found. (Fig. 3, left.) In contrast the intrahepatic bile ducts were markedly dilated (Fig. 3, center) thereby clearly localizing the site of biliary obstruction at the hilus of the liver. Whether it should be called intra- or extrahepatic obstruction is a question of semantics.

The gallbladder was slightly dilated. Its mucosa was intact but puckered in the neck. (Fig. 3, right.) On the cut surface the puckering could be seen to result from deposition of a grey-white somewhat granular tissue contiguous with the areolar tissue of the hepatic hilus. It compressed from the outside the bifurcation of the hepatic duct just where it enters the liver. Microscopically this firm granular tissue which also separated the gallbladder from the liver contained mucus-producing ducts of an infiltrating adenocarcinoma. (Fig. 4.) In the wall of the dilated bile ducts, nests of large cells with foamy cyto-

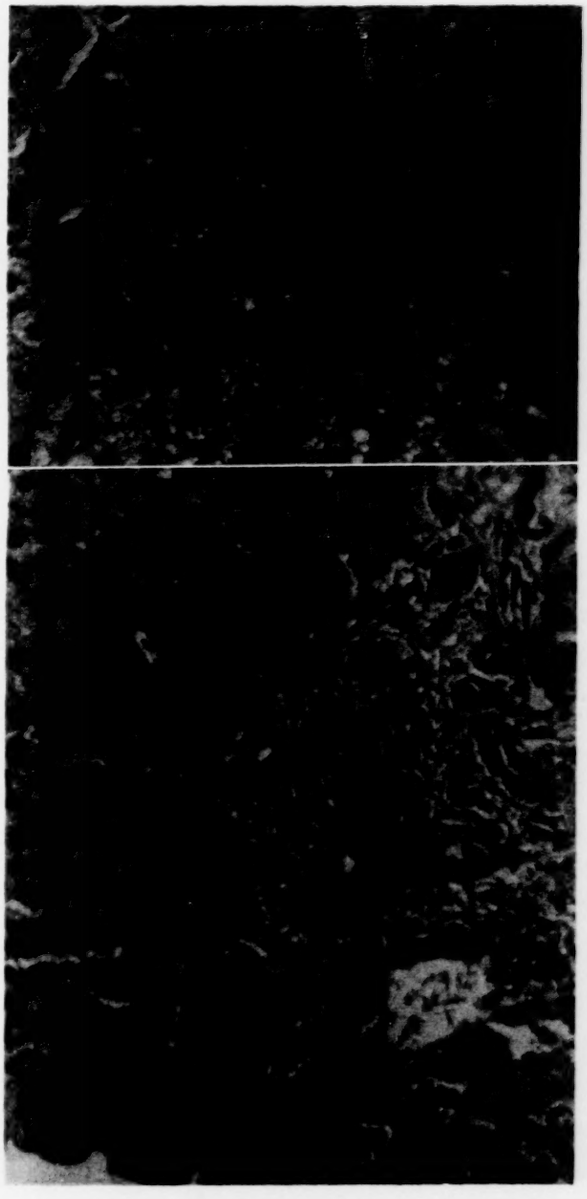

FIG. 1. Biopsy specimen of liver. Top, accumulation of bile pigment in liver cells, Kupffer cells and in bile plugs within bile capillaries around central vein. Rarefaction of cytoplasm results in "feathery" degeneration (arrow). Bottom, circular fibrosis of portal tract which exhibits increased number of septal bile ducts. Bile plug in dilated bile ductule (arrow). Liver cells in lobular periphery not altered. Both hematoxylin and eosin, × 230.

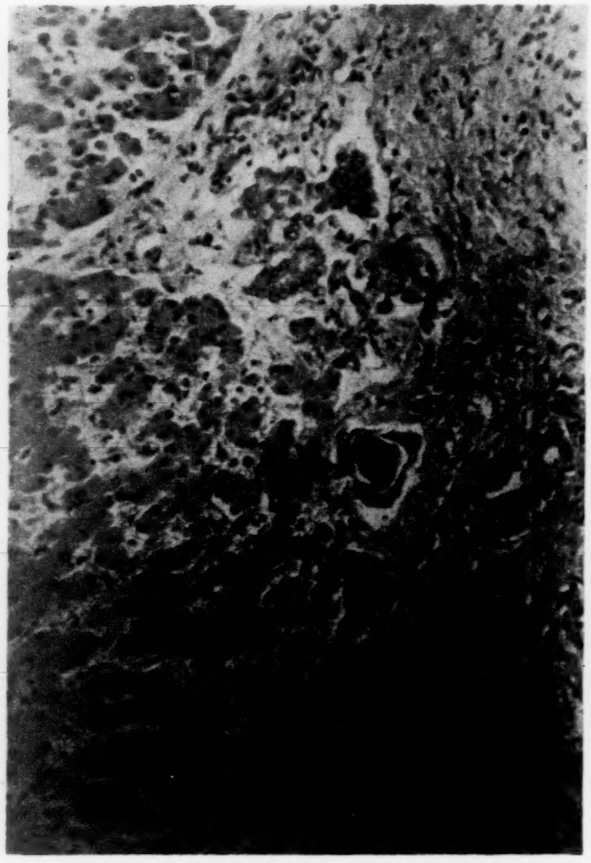

FIG. 2A. Autopsy specimen of liver. Extensive portal fibrosis with conspicuous proliferation of septal bile ducts, some of which contain microcalculi (arrow). Hematoxylin and eosin, × 120.

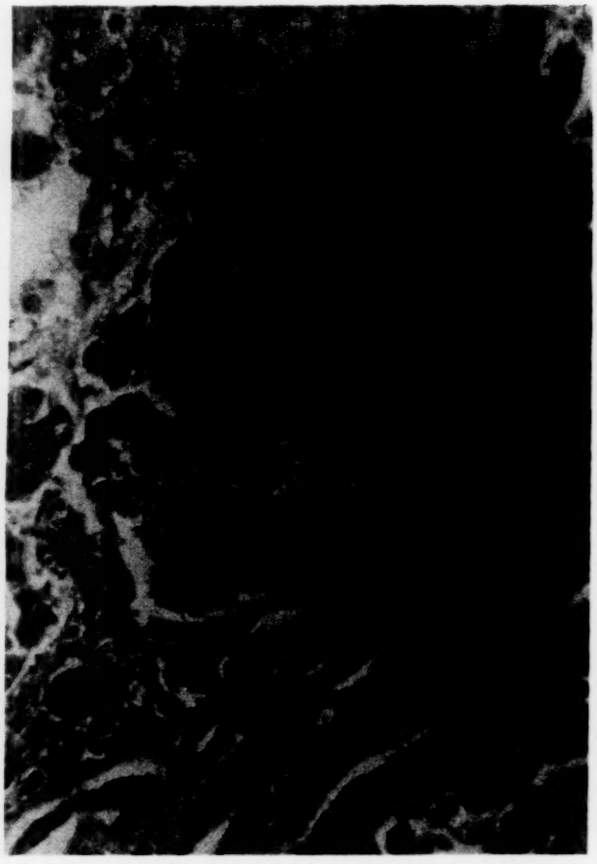

FIG. 2B. Extravasation of bile from ducts or ductules into tissue of portal tract (arrow) surrounded by inflammatory cells. Hematoxylin and eosin, × 230.

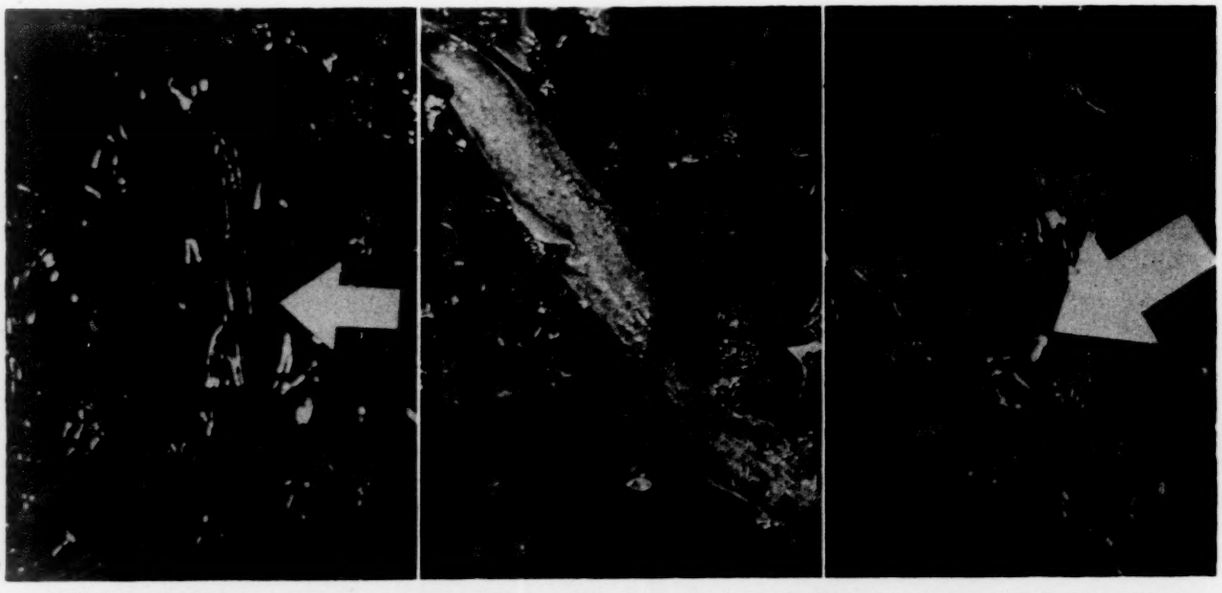

FIG. 3. Left, narrow common duct near papilla of Vater. Center, conspicuously dilated intrahepatic bile duct with deep yellow mucosa. Right, puckering of mucosa of the neck of the dilated gallbladder.

FIG. 4. Mucus-producing adenocarcinoma between cystic duct and gallbladder bed (between arrows). Hematoxylin and eosin; original magnification, × 80.

plasm were found. Their central nuclei were not enlarged and they were obviously not cancer cells especially since they were free of mucus and contained much fat. These cells could be traced from the subepithelial layers of the ducts to the vessels and represent obviously pseudoxanthoma cells related to the hypercholesteremia and account for the yellow color of the mucosa. The question arises whether they result from escape of lipids from the vessels or from the dilated bile ducts. Otherwise the mucosa of the entire biliary tract was intact and therefore the carcinoma on the hilus of the liver had to be considered metastatic. In the search for the primary site, the entire small intestine was found markedly dilated and in the cecum a fungating tumor was found, 3 to 4 cm. in diameter, extending to the ileocecal valve, obviously the primary site. (Fig. 5.) Histologically this tumor was a papillary adeno-

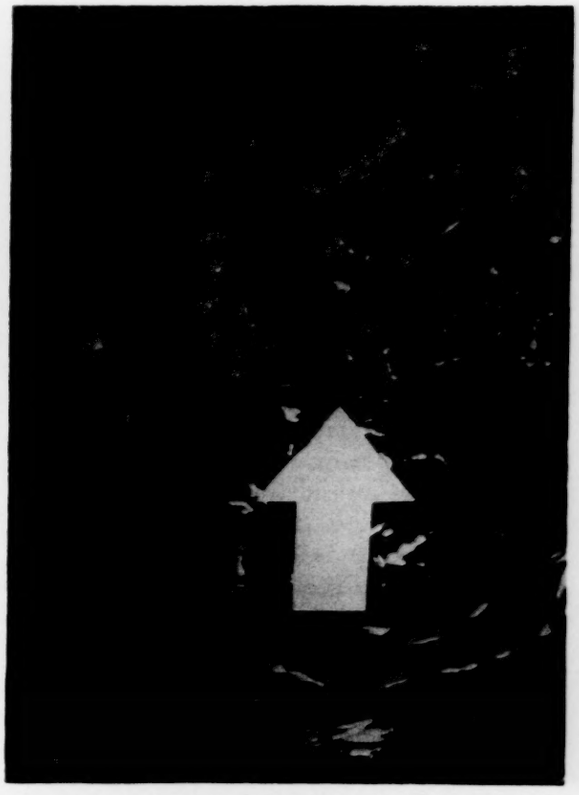

FIG. 5. Polypous carcinoma of the cecum.

carcinoma infiltrating all layers of the cecum, invading veins but not lymph nodes. Carcinoma of the cecum occurs with equal frequency in men and women and the mucus-producing variety, usually very malignant, is not too rare in the younger age group.

Both ovaries were very much enlarged, the right up to 5 by 4 by 3 cm. (Fig. 6A.) On the

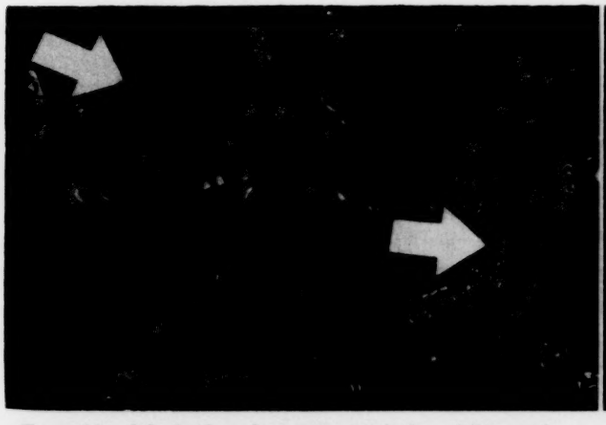

FIG. 6A. Marked enlargement of the right and moderate of the left ovary (arrows) caused by infiltration by mucus-producing adenocarcinoma, metastatic from the cecum.

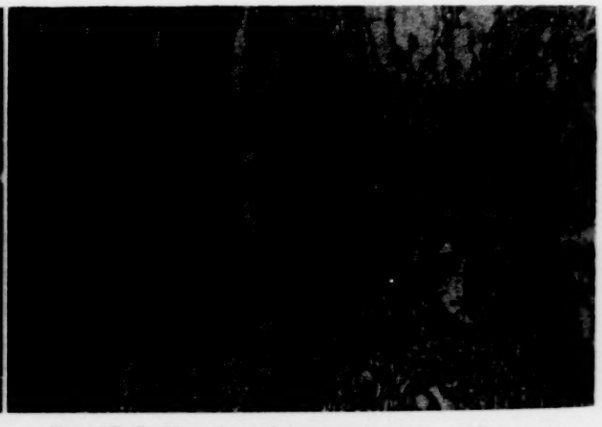

FIG. 6B. Infiltration of the ovarian stroma by mucus-producing adenocarcinoma. Original magnification, × 120.

cut surface they appeared in part cystic but in others infiltrated by a grey-white tissue which microscopically consisted of a mucus producing adenocarcinoma infiltrating the fibrous tissue of the ovary and sometimes being represented by small groups or even single mucus-producing cells. In those cells the nucleus was pushed to the side to produce the signet ring picture, characteristic of the Krukenberg tumor of the ovary which is a frequently bilateral, ovarian metastasis of a gastrointestinal mucus-producing cancer. The organ usually retains its basic shape but becomes variably enlarged. (Fig. 6B.)

The tubes showed some inflammatory changes. The myometrium was infiltrated by adenocarcinoma. Metastases were also found in the wall of the ileum. In the presence of intestinal obstruction, a perforation through such a metastasis resulted in a peritonitis with 3,000 cc. of deep yellow turbid exudate in the peritoneal cavity and fibrinopurulent exudate over the dilated intestinal loops. This development represented the ultimate cause of death.

Attempting a clinical-pathologic correlation, this woman had lower abdominal pain of nine months' duration and the question comes up whether the pain was caused by an independent salpingitis or by the Krukenberg tumor of the ovary. The pain spread and three months before death the carcinoma of the cecum made itself manifest in anorexia, weight loss, bloody diarrhea, fever, some leukocytosis, anemia and hypoproteinemia. To answer the question of the negative x-ray findings, one has to realize that the roentgenologic enema examination was carried out on a severely sick person in whom proper visualization is difficult. Finally, two months before death the metastasis at the hepatic hilus produced obstructive jaundice with hepatomegaly, high serum alkaline phosphatase, absent urinary urobilinogen and normal cephalin flocculation. The high thymol turbidity probably results from the hyperlipemia produced by the biliary obstruction. Eventually intestinal obstruction with biliary peritonitis from a perforated metastasis in the ileum caused abdominal distention and electrolyte imbalance. In conclusion, this case represents a frequent type of disease which produced unusual manifestations.

Final anatomic diagnoses: Mucus producing papillary adenocarcinoma of the cecum causing intestinal obstruction, metastases to the hilus of the liver compressing the hepatic duct and causing biliary obstruction with jaundice, hydrohepatosis and hypercholesteremia with xanthomatous infiltration of the bile ducts; metastases to the ileum with perforation and biliary peritonitis; metastases to both ovaries (Krukenberg tumor) and to the myometrium. Nonspecific chronic salpingitis.

Case Reports

Pituitary Insufficiency*

Diagnostic and Therapeutic Aspects

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Progressive pituitary failure associated with disabling but vague symptomatology may be present for years before a definitive clinical diagnosis of pleuriglandular insufficiency is established. When the basic etiologic lesion is an expanding intra- or extrasellar tumor compressing the optic nerves, optic chiasma or other intracranial tissue, eye signs and symptoms suggestive of a pituitary adenoma may develop early. However, because of the marked variability in location and direction of growth of the tumor in the pituitary gland, a large chromophobe adenoma may occupy the major portion of the sella turcica, and markedly compress or destroy the normal glandular tissue without impinging upon the optic apparatus [*1*]; hence in an appreciable number of cases a valuable early sign may be lost. When acute pituitary necrosis occurs as a result of postpartum hemorrhage and shock the early appearance of gonadal dysfunction (absence of lactation, shrinking of the breast, slow regrowth of hair, amenorrhea) suggests acute pituitary insufficiency [*5*]. However, in the majority of cases of pituitary insufficiency in which the basic pituitary lesion is a slowly progressive fibrosis or atrophy, the symptom-complex developing as a result of slow suppression of pituitary tropic functions is vague and some eleven years elapse, on the average, before suspicion of the lesion is aroused [*2,3*].

Clinical experience has indicated that the gonadotrophic function of the pituitary is the most vulnerable [*1–5*]. Thus gonadal dysfunction may appear within a few weeks following severe postpartum shock. In the menstruating female, absence of lactation and sudden changes in menstruation and in secondary sex characteristics usually arouse suspicion of pituitary involvement [*5*]. However, in the menopausal female, or in the male, gonadal dysfunction manifesting itself as loss of libido or impotence is usually attributed to psychogenic factors. Not until years later, when thyroid and finally adrenocorticoid dysfunction appear, does the diagnosis of pituitary insufficiency become apparent.

It is probably trite to emphasize the importance of early recognition of a pituitary tumor before irreparable damage is done to the visual apparatus and to the pituitary gland itself. A few cases have been reported in which early removal of an expanding intrasellar tumor restored the disturbed vision and endocrine functions [*3*]. The present report concerns one of the unfortunate majority of cases in which the operable pituitary lesion remained unrecognized for many years, seventeen in this instance, before a diagnosis was made. By that time irreparable damage had been done to the optic nerves, chiasma and pituitary functions. The purpose of this case presentation is: (1) to re-emphasize the importance of early diagnosis; (2) to illustrate the essential clinical diagnostic features and laboratory procedures and, (3) to demonstrate the beneficial effects of replacement therapy with desiccated thyroid, testosterone and prednisolone (sterane®).

CASE REPORT

The patient was a fifty-one year old white, unmarried postoffice clerk. Three months prior to his first admission to the hospital he complained of intense chronic headaches, progressive decrease in visual acuity with almost complete blindness of the right eye, moderate fatiguability, loss of libido and potentia, and moderate sensitivity to cold. He consulted his family physician who discovered a secondary

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anemia for which he prescribed iron therapy without apparent relief. Since his visual acuity had been poor for years and was becoming progressively worse, he was advised to consult an ophthalmologist. The latter discovered the patient to have marked decrease in visual acuity, contraction of visual fields and homonymous hemianopsia. He suspected a pituitary tumor and referred the patient to a neurosurgeon who confirmed this diagnosis and advised the patient to enter the hospital immediately for further study and operation.

The patient was admitted to Columbus Hospital on March 6, 1953. At this time his symptoms showed further progression. His exercise tolerance was decreased and he had mild exertional dyspnea but no angina. He had noticed thinning of the hair, especially of the beard area, eye brows and axillas. At the time of admission he had been shaving his beard only once weekly. Enquiry into the functions of the other systems revealed no complaints referable to hearing, nose, throat, mouth, digestion and genitourinary system. Careful interrogation disclosed the fact that decrease in visual acuity was first noticed in 1936 and since then had become progressively worse. Several years following the onset of his defective vision he noticed waning of libido and about five years before this admission he began to notice cold sensitivity. A more complete examination of the eyes revealed homonymous hemianopsia, bilateral optic atrophy which was complete on the right and incomplete on the left, divergent strabismus, and contraction of visual fields. An x-ray film of the skull showed the sella turcica to be enlarged but the clinoid processes were intact. The heart and lungs were normal. The liver and spleen were not palpable. All reflexes were sluggish and none were abnormal. The skin appeared pale, with marked loss of pigment, somewhat dry but with normal turgor. The hair on the beard area was thin and scanty. The penis and testes were hypoplastic. The blood pressure was 110/60; the pulse 90 per minute and regular in rate and rhythm. Routine laboratory work-up showed a hemoglobin of 54 per cent, with a red blood count of 3.9 million cells per cu. mm., a white blood count of 8,900 per cu. mm. and a normal differential count. The serum glucose was 80 mg. per cent, the nonprotein nitrogen 34 mg. per cent. The urinalysis and serologic tests were negative. The electrocardiogram showed low voltage and several non-specific abnormalities.

The patient underwent surgery on March 9, 1953. After exposing the pituitary area the neurosurgeon noted a large pituitary tumor, bluish in color, semisolid in consistency and enveloped in a highly vascular capsule. There was marked thickening of the right optic nerve and compression of the left optic nerve and optic chiasma. A small aperture was made in the capsule and the tumor was removed piecemeal by rongeur and suction. A small amount of intracapsular bleeding was controlled by gelfoam.® The patient's condition was good throughout the operation. His postoperative course was uneventful and he was discharged from the hospital on April 1, 1953. The first histopathologic report of the tumor tissue stated that a fetal carcinoma with precancerous changes were found. Because of this finding and the fact that the neurosurgeon was unable to remove the adenoma completely the patient was advised to return to the hospital for radiation of the pituitary area. Radiation was administered through four portals; each was 6 by 4 cm. (24 sq. cm.). These portals were directed to the pituitary area by means of the pin and arc device to insure oblique beams hitting the tissue target. Each portal received a total of 3,138 r on the skin daily for twenty-five days. The total tumor dose was calculated to be 5,350 r.

At a later date, sections of the tissue removed at operation were subjected to a more intense and critical study by several observers. The conclusion reached was that the tumor was a chromophobe adenoma with fetal cell rests.

In the two years following the operation and x-ray radiation the patient's headaches disappeared, vision of the right eye was completely lost, and the left eye had only partial vision. His previous symptoms progressed. He had gained considerable weight and had developed a eunuchoid body build. He was readmitted to the hospital on June 6, 1955, for a complete endocrine appraisal and treatment. Apart from upper respiratory infections, the patient had no major illnesses between the first and present admission.

On physical examination, the patient's skin was waxy pale with noticeable decrease in pigment, especially at the areolas of the nipples. The facies appeared myxedematous. The eyebrows, axillas and pubic regions showed a moderate thinning of the hair. The hair distribution of the pubic region was that of the typical female escutcheon. Examination of the eyes showed the pupils to be round and equal, and they reacted to light and accommodation. The funduscopic examination disclosed complete atrophy of the optic nerve on the right and partial atrophy on the left, without quantitative change from previous examinations. The ears, nose and throat were normal. There was no cervical lymphadenopathy and no palpable thyroid. The heart appeared normal in size and rhythm with a grade 1 systolic murmur at the apex. The lungs were clear. The abdomen was scaphoid in contour and the liver and spleen were not palpable. No masses or tenderness were noted. Examination of the inguinal regions revealed enlarged rings but no hernia. The testes and penis were moderately hypoplastic and the general body build was eunuchoid in appearance. The extremities were long and slender with an abnormal amount of fat deposited in the buttocks and thigh regions. All reflexes were normal but sluggish.

Routine urinalysis revealed no abnormalities. The

blood showed a hemoglobin of 57 per cent with a red blood count of 3.1 million per cu. mm. The white blood count was 10,100 per cu. mm. with a normal differential count. The serum calcium was 10.8 mg. per cent and the serum cholesterol 390 mg. per cent with 230 mg. per cent cholesterol esters. The serum non-protein nitrogen was 30 mg. per cent. The total serum proteins were 6.0 gm. per cent, albumin 4.0 gm. per cent and globulin 2.0 gm. per cent. Serologic tests were negative. A fasting blood sugar level was 104 mg. per cent. X-ray of the chest revealed the heart to be normal in size and the lungs clear. The electrocardiogram showed low voltage and many non-specific abnormalities.

The insulin tolerance test, employing 0.1 unit of regular insulin per Kg. body weight (6.5 units) disclosed a hypoglycemic unresponsiveness.

INTRAVENOUS INSULIN TOLERANCE TEST

Time (min.) . .	0	10	20	30	45	60	90	120
Blood sugar (mg. %) . . .	104	87	53	40	34	46	54	68

The radioiodine uptake was 14.5 per cent with a conversion ratio of 4.1 per cent. Following the administration of thyroid stimulating hormone (TSH) the radioiodine uptake rose to 57 per cent with a conversion ratio of 26 per cent. The basal metabolic rate was minus 40 per cent of normal. The eosinophil count was 250 cells per cu. ml. and after ACTH (40 units) stimulation the count dropped to 150 cells per cu. ml. The 17-ketosteroid excretion was 4.0 mg. per twenty-four hours. Serum sodium was 140 mEq./L.; chlorides 98 mEq./L. and potassium 4.5 mEq./L. Several testicular biopsies yielded chiefly fibrous tissue. It was believed that these samples were derived from the tunica vaginalis and did not represent testicular tissue proper.

The final diagnosis was (1) chromophobe adenoma with fetal cell rests; (2) pituitary insufficiency with progressive gonadal, thyroid and adrenocortical failure; and (3) blindness of the right eye, partial blindness of the left eye, and homonymous hemianopsia.

The patient was given a high protein and high salt diet. Oral prednisolone (sterane) was prescribed in 5 mg. dosage twice daily and testosterone was given in 12.5 mg. dosage daily. After one month on this regimen he was given thyroid, 30 mg. daily, which was increased to 0.2 gm. daily in the ensuing three months.

The effect of the hormone therapy was indeed gratifying in that the patient regained his sense of well being, strength, vigor and libido. He now shaved every day, which had a good psychologic effect on his ego. His myxedematous facies became less pronounced and the cold sensitivity disappeared. Routine laboratory work-up showed the basal metabolic rate to be minus 20 per cent of normal; cholesterol 250 gm. per cent; a normal hemogram and urinalysis. However, his skin still revealed a conspicuous absence of pigment.

COMMENTS

The presence of a chromophobe tumor may remain clinically unrecognized for years until one of several manifestations first becomes evident: (1) progressive visual defect—decrease in visual acuity, contraction of visual fields and homonymous hemianopsia; (2) hormonal deficiency—particularly gonadal dysfunction followed by suppression of other pituitary tropic hormones, manifested as thyroid and adrenocortical insufficiency. Occasionally, skull x-rays taken during the routine investigation of a headache may reveal an enlarged sella turcica which may stimulate further search for a pituitary tumor. Depending upon the direction of growth, a chromophobe tumor may attain a large size before producing recognizable clinical features [*1*] or, in some instances, produce bizarre neurologic symptoms unassociated with hormonal or visual dysfunctions.

The patient herein described complained of progressive failure of vision, headaches and scotomas for seventeen years before suspicion of a tumor was aroused. Several years following the onset of defective vision he began to complain of symptoms suggestive of gonadotropic dysfunction, characterized by loss in libido, potentia and change in body build, which were interpreted as manifestations of chronic anxiety and depression. Five years before he was operated upon for a chromophobe tumor, symptoms which were characteristic of severe hypothyroidism developed. Since a secondary anemia was discovered at this time, the attending physician interpreted the fatigue and cold sensitivity as due to anemia. By the time the patient had lost almost complete vision of the right eye and partial vision of the left, he was advised to consult an ophthalmologist, who made the diagnosis of a pituitary tumor. The unfortunate aspect of this case was the finding at operation of irreparable destruction of the right optic nerve and marked pressure upon the left optic nerve and optic chiasma. The tumor was very large and encapsulated.

Pituitary adenomas comprise 7 per cent of all intracranial tumors [*6*] and of these 76.9 per cent are chromophobe adenomas. This type of adenoma usually begins as a small discrete collection of chromophobe cells, usually encapsulated [*7*], and may range from microscopic

size to 0.5 cm. in diameter. The normal pituitary gland occupies only about 50 per cent of the volume of the sella turcica, consequently a chromophobe adenoma may expand to occupy the remaining portion of the sella and yet not produce recognizable symptoms. Depending upon the location of the adenoma in the sella turcica, the direction of its dynamic growth determines the hormonal and neurologic signs and symptoms. For instance, the tumor may expand in the sella and destroy by compression a considerable portion of the pituitary gland, or its dynamic growth may extend upward, stretch the diaphragma sella and compress either the optic nerves or chiasma, without affecting pituitary tissue to any appreciable extent. In this connection it is of interest to note that the pituitary gland has a remarkable factor of safety, since a considerable amount of normal glandular tissue must be destroyed before pleuriglandular signs and symptoms become evident [*1*]. Should the direction of growth be toward the floor of the sella, it may break through and invade the sphenoidal sinus. Cases have been reported [*1*] in which the tumor had expanded into the floor of the third ventricle to produce internal hydrocephalus or into the temporal lobe and remained dormant for years. The chromophobe tumor of the present case was demonstrated at operation to have a smooth, highly vascular capsule after it had broken through the diaphragma sella. Such encapsulation of the tumor prevents it from infiltrating into surrounding tissue and usually facilitates its removal. Encapsulated chromophobe tumors have been previously reported [*8*].

In the majority of cases a chromophobe adenoma produces well recognized signs of compression of the optic apparatus. Should the tumor impinge upon one optic nerve, partial or complete blindness may occur in the corresponding eye without affecting the other. Both optic nerves may be compressed by a tumor of large size, producing total blindness. Should, however, the tumor expand laterally out of the sella and compress the optic tract behind the optic chiasma, then homonymous hemianopsia occurs. This was the situation of the tumor in the present case in which destruction of the right optic nerve and marked compression of the optic chiasma and optic tract behind it occurred.

The earliest symptoms produced by the chromophobe tumor are usually suggestive of a psychosomatic disturbance. The fundamental pathologic physiology of the early symptom-complex is difficult to explain. However some investigators [*1*] have attempted to interpret these symptoms on the basis of cerebral and hypothalamic compression. The headaches and visual disturbances are caused by the expanding intracranial tumor and are usually the two symptoms which induce the patient to seek medical advice. The earliest endocrine symptoms are suggestive of gonadal insufficiency. It is generally agreed that gonadotropic function of the pituitary is most vulnerable to compressive destruction of the tumor [*2,3,5*]. Thyroid function is usually next affected, ordinarily years later, and manifests itself in clinical features of hypothyroidism [*4*]. Adrenocortical function suffers least and is longest preserved [*3,4,8*]. Cases have been reported in which gonadal function was completely abolished without impairment of the visual apparatus, thyroid or adrenal cortex [*4*].

Assessment of endocrine dysfunction is of prime importance in the diagnosis of a pituitary tumor. This is especially true in cases in which there is no visual disturbance and only clinical features of endocrinopathy are present. The function of the pituitary gland *per se* was estimated by the use of the intravenous insulin tolerance test. This clinical test was first introduced by Fraser and Smith [*8*] in 1941 for the evaluation of pituitary dysfunction and was extensively employed by Sheehan et al. [*5*] in their classic investigation of hypopituitarism following postpartum hemorrhage. Although the recommended dose is 0.1 unit of regular insulin per kilo, as was employed in the present investigation, it is believed by most workers [*5*] that a dose of 0.03 unit of insulin is quite adequate, much safer and less likely to cause alarming insulin reactions. Demonstration of hypoglycemic unresponsiveness with this test in the present case supported the impression of considerable pituitary destruction. Fraser, Albright and Smith [*9*] compared the insulin tolerance curves of normal persons with those in persons with myxedema, pituitary insufficiency and Addison's disease. They found that only adrenal insufficiency produced a curve simulating pituitary insufficiency, but the curves for patients with Addison's disease did not drop so precipitously; however, the hypoglycemic unresponsiveness lasted longer. More recently, the same authors expressed the belief that the insulin tolerance test is the only one which will separate

the myxedema of pituitary origin from primary hypothyroidism. There is still some controversy regarding the most sensitive tests for thyroid function [*4*] in the presence of pituitary insufficiency.

In the present case, secondary hypothyroidism was diagnosed by measuring the radioactive iodine uptake before and after stimulation of the pituitary gland with TSH. These studies showed a radioiodine uptake of 14.5 per cent before, and 57.0 per cent uptake following the administration of TSH. This procedure has been well worked out and amplified by Bishopric et al. [*10*]. If hypothyroidism is secondary to pituitary failure, the thyroid gland is structurally and potentially functionally normal and a normal response with TSH is expected, as was demonstrated by the present case. The basal metabolic rate was minus 40 per cent of normal and the cholesterol was 390 mg. per cent. The adrenocortical function was least affected, as has been the experience of other workers. There was only a slight disturbance in electrolyte equilibrium, which confirms the observations by other investigators [*11*] who showed that secretion of aldosterone continues, probably at a lower level, in the absence of pituitary function. The 17-ketosteroid excretion was 4 mg. per twenty-four hours (average normal range is 8 to 20 mg. per twenty-four hours), which suggests moderate suppression of pituitary corticotropic function. The drop in eosinophil count following administration of ACTH was less than 50 per cent, which again supports the diagnosis of adrenocortical insufficiency.

The treatment of pituitary insufficiency caused by a chromophobe adenoma consists of surgery or radiation, or a combination of these two procedures in addition to replacement hormonal therapy. One of the major aims is to arrest the progressive visual defect either by surgery or by radiating the expanding tumor with the hope of restoring at least part of the lost vision. Unfortunately, in the present case the tumor had been present for many years with consequent irreparable damage to both eyes. The question whether to radiate or operate depends upon the presence or absence of pressure on the optic apparatus. Horrax and associates [*12*] have adopted the criterion that, in cases in which the chief symptoms are attributable only to pituitary insufficiency without signs of visual impairment, radiation is indicated, at least for the time being. Should signs of visual impairment become evident, surgery is the rational course. However, cases have been reported [*3*], and are mentioned by Horrax et al. [*12*], in which removal of the chromophobe adenoma, even in the absence of visual defect, has had a salutary effect on pituitary tropic functions in many instances. In the present case the patient received radiation therapy following surgery. This was due to the fact that the neurosurgeon had been successful only in removing the greater part of the extrasellar extension of the chromophobe tumor. Furthermore, there was some suspicion that the tissue removed at operation contained malignant cells. The patient was not treated with ACTH or glucocorticoids before or during the operation, to prevent adrenal crisis, but he withstood the stress of the operation quite well anyway and made an excellent recovery. Although the patient had outstanding symptoms of severe pituitary insufficiency, endocrine function was not evaluated or hormonal therapy given until two years following the operation. The patient's endocrine status was then evaluated and he was given replacement hormonal therapy: thyroid, testosterone and prednisolone (sterane). He made a remarkable recovery in three months on this regimen. Desoxycorticosterone (DOCA) was not included in the treatment since his serum electrolytes did not vary from the normal to any extent. It was felt that the electrolyte balance could be maintained by administering a high salt diet. Horrax et al. [*12*] pointed out that several of their patients with pituitary insufficiency, who were placed initially on DOCA, were well maintained in electrolyte balance when DOCA was later discontinued. The patient failed to regain any of the lost skin pigment in spite of one year of intensive hormonal treatment. This phenomenon may be explained by the absence of MSH (melanin-stimulating hormone) normally elaborated by the pituitary gland [*13*]. The important aspect of the management of this case was the restoration of a patient to a gainful livelihood.

SUMMARY

1. A case is presented of pituitary insufficiency caused by a large pituitary adenoma.

2. The clinical diagnostic aspects and assessment of endocrine function are discussed. The causes of unfortunate delays in diagnosis and treatment, sometimes with loss of vision and other disabilities, are considered.

3. The treatment consisted of surgical removal of the tumor, radiation and hormonal replacement by thyroid, testosterone, prednisolone (sterane) and a high salt diet.

Acknowledgment: We wish to thank Dr. M. William Amster of Chas. Pfizer and Co. for supplying all the hormones used in this study.

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Staphylococcus Bacterial Endocarditis*

Report of a Case in a Narcotic Addict

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THIS case is reported to demonstrate the difficulties encountered in the treatment of staphylococcus bacteremia and to re-emphasize the extreme degree of antibiotic resistance of many strains of staphylococci, as well as to add an additional report of bacterial endocarditis in a narcotic addict to the literature.

The patient, a thirty-four year old surgeon and World War II veteran, was admitted to Winter Veterans Administration Hospital on August 17, 1955, for treatment of a drug addiction of approximately six months' duration. The past history is relevant only in that he had a hearing loss in the left ear and that there had been, while in the Service, an episode of what was thought to be transient auricular fibrillation. There was no other history suggesting rheumatic disease.

Early in 1955, he developed an addiction to dilaudid.® In April, he consulted a psychiatrist and achieved temporary withdrawal. A month later he was again using narcotics, employing his femoral veins for each injection. In mid-July, he had edema of the legs which was more marked in the right leg. Two weeks prior to our examination he injured the right anterior chest wall in an automobile accident. On August 13, he was admitted to another hospital where a diagnosis of right lower lobe pneumonia, bilateral iliofemoral thrombophlebitis and narcotic addiction was made. The patient had a temperature of 104°F. and an anemia for which he received two transfusions. He was started on a drug-withdrawal program and given a combination of penicillin and streptomycin.

On August 17, the patient was transferred to Winter Veterans Administration Hospital. The examination on admission disclosed an anxious young man whose legs were in pressure bandages. His temperature was 100.2°F., pulse 90 and respirations 20. There was complete deafness of the left ear and partial hearing in the right. The right thorax was tender over its entire anterior aspect. Dullness, diminished breath sounds and scattered moist rales were found in the right lower posterior axilla. The heart was not enlarged; no murmurs were heard. The liver edge was just below the costal border. Both legs showed pitting edema to the knees, which was more pronounced in the right leg, with raised, keloid-like, pea-sized injection sites overlying both femoral veins. There was no tenderness in either the femoral or calf muscle.

On admission the hemoglobin was 9.1 gm. per cent with 3.4 million erythrocytes, 16,200 leukocytes and 93 per cent neutrophils per cu. mm. The urinalysis was negative; two days later the sediment contained occasional red cells and granular casts. An electrocardiogram showed a sinus tachycardia. The chest x-ray taken on admission was clear; two days later a small area of increased density was seen in the anterior region of the left third rib. Subsequent chest x-rays were negative.

The patient was continued on the penicillin-streptomycin combination which he had received prior to admission. Methadon was substituted for the dilaudid; complete narcotic withdrawal was contemplated. A psychiatric consultant was called, and plans for the patient's transfer to a psychiatric service to follow up recovery from his medical illness were formulated.

On his third hospital day the patient had a chill with a temperature rise to 104°F.; the following day his temperature reached 105°F. For the first time a roughened systolic murmur was heard in the fourth left interspace adjacent to the sternum which transmitted downward into the epigastrium and to the left, but did not reach the axilla. The spleen was not felt. Three petechiae were seen on the left great toe and one on the right. At this time a blood culture was obtained and a gram-positive coccus reported. The organism showed *in vitro* sensitivity to chloram-

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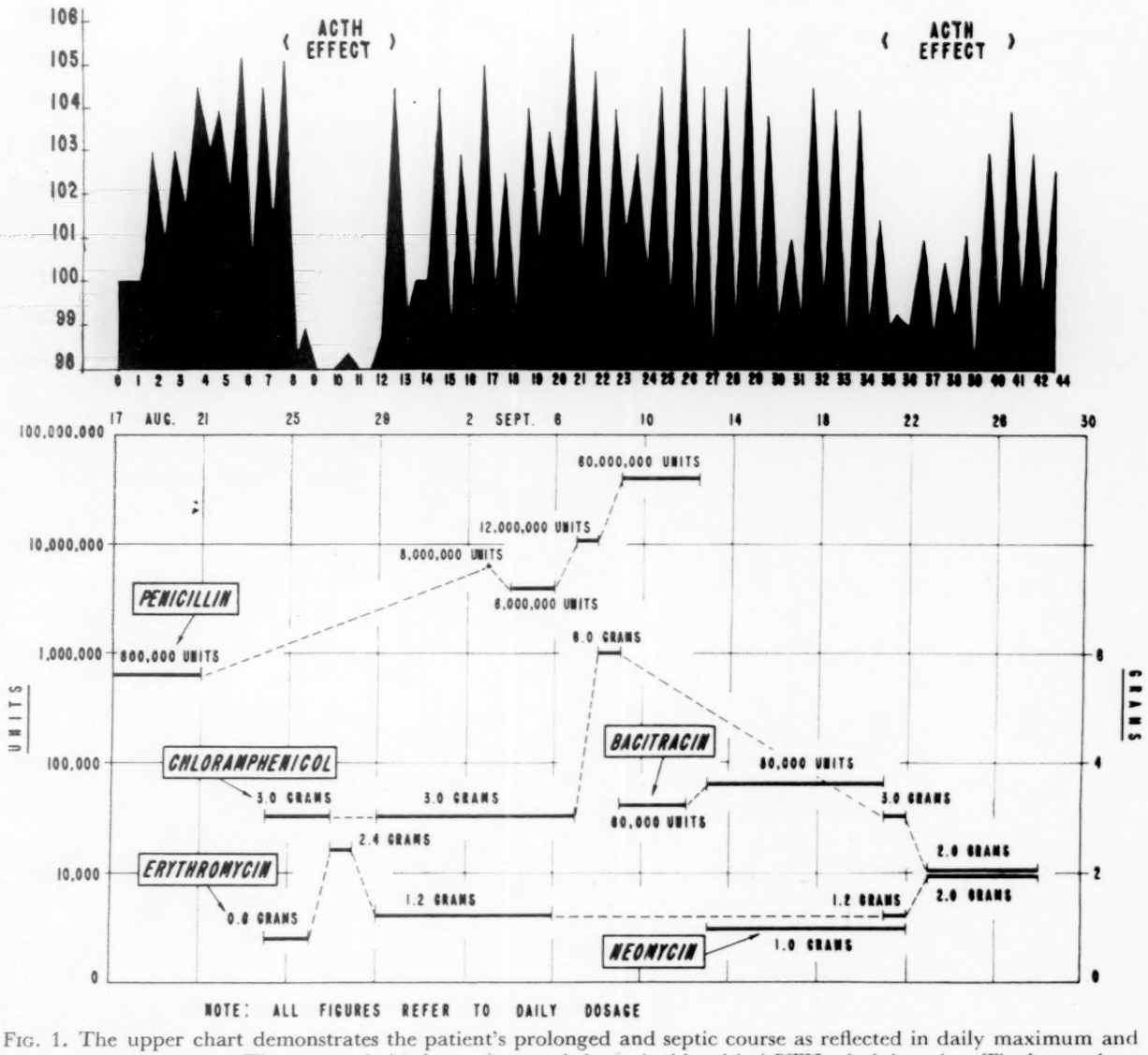

FIG. 1. The upper chart demonstrates the patient's prolonged and septic course as reflected in daily maximum and minimum temperatures. The two periods of apparent remission coincide with ACTH administration. The lower chart portrays antibiotic dosage.

phenicol, erythromycin and furadantin,® but none to penicillin or to the tetracycline group. This same organism was subsequently obtained on blood culture on each of eleven occasions. In no culture did this organism fail to grow.

While awaiting the first report on the blood culture, the penicillin-streptomycin combination was stopped and tetracycline briefly administered. Following the report of a coccus (first thought to be an alpha hemolytic streptococcus) administration of 3.0 gm. of chloramphenicol and 2.4 gm. of erythromycin was started orally. Chloramphenicol dosage was then increased to 6.0 gm. daily. On August 25, the patient had a chill and a temperature of 105°F., the sixth such episode in five days; he appeared moribund, with Cheyne-Stokes respirations. Because of his shock and high fever he was given ACTH; his temperature sharply dropped to a subnormal level. This level was maintained for four days until ACTH was significantly reduced. (Fig. 1.)

In vitro, the coccus was consistently sensitive to chloramphenicol, erythromycin, furadantin, neomycin and bacitracin. Despite apparent streptomycin insensitivity, streptomycin was used in conjunction with nearly all the penicillin he received in the hope of attaining a clinical synergistic effect. Furadantin, although primarily useful in urinary tract infection, was administered in amounts of 1.2 gm. daily for a total of nineteen days in the hope of achieving

a systemic effect. Late in the course of his illness chloramphenicol and erythromycin were re-administered, this time by the intravenous route in amounts to ensure 2 gm. of each in twenty-four hours.

Partial suppression of growth of the organism *in vitro* by penicillin in amounts corresponding to 1,000 units per cc. agar broth was demonstrated. Therefore 60 million units of penicillin were administered daily by constant intravenous infusion in conjunction with benemid® (2.0 gm.). This was in addition to the intramuscular chloramphenicol (6.0 gm.) and streptomycin (2.0 gm.) which he was receiving. The patient's septic course continued unabated.

At the end of his third week in the hospital bacitracin was started in amounts of 60,000 units daily. This was later increased to 80,000 units for a total of twelve days. Neomycin (1.0 gm.) was given daily for nine days with no effect other than an apparent increase in the nausea and vomiting which earlier had seriously interfered with the administration of oral antibiotics. Blood cultures remained positive. The patient's progressive anemia was controlled by six blood transfusions. Bacitracin had been started on September 11. Repeated urinalyses after September 12 showed increasing numbers of erythrocytes and leukocytes in the urine and an increase in albuminuria and casts. On September 16, the blood urea nitrogen was 15 mg. per cent; by September 27 it had increased to 67 mg. per cent. Generalized anasarca, partially attributable to ACTH which was administered between September 15 and 25, was paradoxically associated with a serum sodium of 126 mEq./L., a potassium of 5.6 mEq./L. and a mild hypoproteinemia, and further evidences of renal insufficiency. The total protein was 6.4 gm. per cent with 3.8 gm. of albumin and 2.6 gm. of globulin on September 26.

Increasing distention of the neck veins became apparent, along with a tremendous enlargement of the spleen and liver and an hepatojugular reflux. The murmur became increasingly intense and on September 26, he was digitalized. Kussmaul breathing developed on September 27 and the patient died in the coma of renal failure on September 28, forty-two days after his admission to the hospital.

Autopsy Data. At autopsy the heart weighed 350 gm. Positive findings were limited to the tricuspid valve which was dilated to 12.5 cm. as compared to the mitral measurement of 10 cm. The posterior leaf of the valve was eroded;

FIG. 2. Vegetations involving the tricuspid valve.

attached to its base was a large, uniformly white, friable vegetation which was 3 cm. wide at its base and had two projecting arms 2 cm. and 1½ cm. in length. The other valves were uninvolved. No gross or microscopic evidence of pre-existing heart disease was found. (Fig. 2.)

The lungs showed complete occlusion of a main branch of the right pulmonary artery by a pale and tightly attached embolus which was similar in appearance to the tricuspid vegetation. Throughout both lungs smaller arterioles were blocked by fresher thrombotic material. Below the inguinal ligament the dissected right femoral vein showed fibrosis and scarring, forming an apparently completely occluded tough and fibrous cord. The left femoral vein was patent as far as the inguinal ring.

The spleen was huge, weighing 1,000 gm. It was moderately soft and swollen. The liver was likewise enlarged, weighing 3,150 gm. The large pale pink kidneys showed numerous tiny focal hemorrhages, giving them a flea-bitten appearance. The cortices were swollen and the purplish red pyramids stood out in sharp contrast to the neighboring tissue. The microscopic sections of the kidneys seemed to show a combination of focal embolic glomerulitis, glomerulonephritis and tubular damage which is possibly due to nephrotoxic agents.

COMMENTS

The tricuspid valve has been the site of predilection for vegetations in patients in whom bacterial endocarditis has been attributable to intravenous injection of contaminated heroin. Hussey and Katz have reported 102 patients who had infections resulting from narcotic addictions. Of this group four cases of acute bacterial endocarditis came to autopsy. In three of these, Staphylococcus aureus was the causative organism and the tricuspid valve was the site of implantation. In the remaining case the aortic valve was involved and the responsible agent was Bacillus pyocyaneus. Hussey and Katz reported an additional series of four cases in which the diagnosis of endocarditis of the right side of the heart was established clinically on the basis of a triad consisting of evidence of addiction (heroin), septicemia with positive blood cultures, and x-ray evidence of pulmonary infarction in the absence of disease in the peripheral veins. Heart murmurs were not prominent in the group which they reported. There was no evidence of preexisting valvular disease in any of their cases [*1*].

Our patient may have been unique in that injection was limited to the femoral veins and to the same small area of these veins so that keloid formation was evident. The repeated irritation of the vein walls and perivenous tissues produced a gradual throttling of the femoral veins along with indolent edema of the lower extremities which gave the appearance of a chronic thrombophlebitis.

The tricuspid valve vegetations in our case were presumably the source of the emboli found throughout both lungs. The largest embolus, involving a main branch of the right pulmonary artery, was identical in appearance with the greyish white tricuspid vegetations and contained many bacteria in the microscopic sections. Transitory shadows which were seen in the chest x-ray shortly after the patient's admission were thought to be small pulmonary infarcts. The original "pneumonia" which cleared so rapidly probably represented an infarction.

In the past few years numerous reports of penicillin-resistant strains of staphylococci have appeared; the percentage of penicillin-resistant strains isolated from patients in larger hospitals of various parts of the world has steadily increased. In most hospitals 75 per cent of strains of staphylococci are penicillin-resistant. Micrococci from nose and throat cultures of hospital personnel are also penicillin-resistant in similar proportions, Finland notes [*2*]. As other antibiotics have come into extensive use the emergence of resistant micrococci has followed within an incredibly short period of time. Finland remarks on an experience gained at a Chicago hospital where "the extensive use of erythromycin has recapitulated within a brief period of time the longer experience with penicillin. The extensive use of this agent led to an increase in the incidence of erythromycin-resistant micrococci among hospital personnel of from 0 per cent to 75 per cent within a period of five months" [*2*].

The professional and hospital background in our patient makes it easy to understand the penicillin and streptomycin insensitivity which the invading organism showed. In the laboratory we were continuously able to demonstrate sensitivity of this staphylococcus to concentrations of antibiotics easily achieved in the patient's body fluids, nevertheless at no time did we sterilize the blood stream or produce even temporary clinical improvement despite what seemed to be adequate dosage. Whether the bacteria were too firmly entrenched behind a dense layer of fibrin on the tricuspid vegetations to be reached by attainable antibiotic concentrations, or whether they achieved some resistance in the tissues which could not be demonstrated in culture are questions which could not be answered.

SUMMARY AND CONCLUSIONS

1. A fatal case of staphylococcus endocarditis developing in a young surgeon after self-administration of narcotics is presented.

2. This case is similar to three earlier cases in which autopsy findings have been reviewed by Hussey et al. These patients present a constellation of narcotic addiction, staphylococcus bacteremia and bacterial endocarditis limited to the tricuspid valve.

3. The staphylococcus in our case was highly resistant to penicillin and to streptomycin. This resistance may have been directly related to the patient's professional and hospital background.

4. In spite of *in vitro* sensitivity to chloramphenicol, erythromycin, bacitracin and neomycin, neither temporary clinical improvement nor blood stream sterilization was achieved. Death was due to renal failure.

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The Coincidence of Hemoglobin J and Fanconi's Syndrome of Hypoplastic Anemia with Hypoplasia of the Spleen in a Young Man*

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HEMOGLOBIN J has recently been reported as a seventh type of abnormal hemoglobin [*1*]. Congenital splenic aplasia has been recorded in over thirty patients, usually associated with other congenital abnormalities [*5–7*]. Only four examples have been reported of Fanconi's hypoplastic disease of the bone marrow occurring in adults [*8,9*]. Recently, at Walter Reed Army Hospital, a twenty year old white male patient was studied who demonstrated all three of these abnormalities. It is the purpose of this paper to present a report of this unusual case together with some pertinent observations on members of the patient's immediate family.

METHODS

Platelet counts were done by the technic of Brecher and Cronkite [*10*]. Hemoglobin paper electrophoresis was performed by a method developed in this laboratory [*11*]. Hemoglobin solubility studies were performed by Dr. Harvey Itano employing a method which he has previously described [*12*]. The remaining procedures were performed in the usual manner.

CASE REPORT

A twenty year old white soldier was admitted to a military hospital on July 9, 1955, complaining of large tender bruises. Three weeks prior to admission painless, non-pruritic areas of purplish discoloration developed over his lower legs. These lesions spread to involve the thighs. Simultaneously, generalized fatigue developed. There was no history of similar episodes previously and the patient had not been exposed to any hemotoxic agents. Physical examination revealed petechial lesions in the oropharynx and areas of purpura scattered over both legs and thighs. On cardiac auscultation there was a grade 2 blowing murmur in the pulmonic area. Initial laboratory studies revealed a white blood cell count of 5,500 per cu. mm. with a normal differential, a red cell count of 3,200,000 per cu. mm. and a hemoglobin of 12.8 gm. per 100 ml. The platelet count was 41,140. The bleeding time was four minutes and the coagulation time eight minutes. Prothrombin activity was 100 per cent and clot retraction, absent at six hours, was complete at twenty-four hours. An L.E. cell preparation was negative. A sternal marrow aspiration showed almost complete absence of megakaryocytes, without other abnormalities. A diagnosis of "thrombocytopenia due to undetermined cause" was made and the patient was started on treatment with cortisone orally, 100 mg. daily. One week after admission the cortisone was increased to 400 mg. daily because of an episode of rectal bleeding and gross hematuria. Peripheral smears revealed no platelets at that time. After ten days the gross bleeding ceased and no further hemorrhages occurred. However, the patient began to experience substernal chest pain radiating down the inner aspects of both arms, precipitated by effort and relieved promptly by rest. An electrocardiogram revealed non-specific T wave abnormalities. Because of the lack of response to cortisone therapy the patient was transferred to Walter Reed Army Hospital on September 23, 1955, for further observation. On arrival his only complaints were fatigue and substernal pain on effort.

The patient had always been in good health except for frequent respiratory infections. He had been hospitalized in December, 1953, for observation because of fatigue. No disease was found and laboratory

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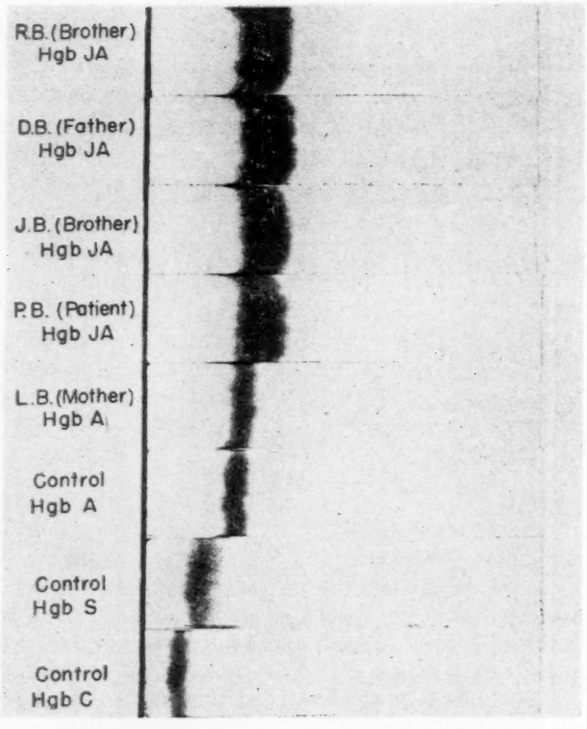

FIG. 1. Paper electrophoresis showing the hemoglobin pattern of the patient and members of his family. The patient, his father and two brothers have hemoglobin A-J. His mother has hemoglobin A.

studies performed at that time revealed a leukocyte count of 9,900 per cu. mm., red cell count of 5,000,000 per cu. mm., hemoglobin of 15.6 gm. per cent and a normal red cell morphology. The patient's parents and four brothers were in good health. His mother, maternal uncle and maternal cousin had strabismus.

Examination revealed a well developed, alert but slightly pale white man. Examination of the eyes disclosed an internal strabismus on the right with only light perception on the affected side. Ocular muscle power and vision were normal on the left. Examination of the skin and mucous membranes revealed pigmented residuals of purpura over both legs. A few purpuric lesions were present on the right thigh and in the left antecubital fossa. Cardiovascular examination revealed a normal rhythm, no cardiomegaly, blood pressure 120/50 bilaterally. There was a harsh systolic murmur located at the left sternal border and transmitted into the pulmonic area. No diastolic murmurs were heard. The liver and spleen were not palpable.

Laboratory studies: Urinalysis was normal. Serologic tests for syphilis were negative. Serum lipids were 865 mg. per 100 ml. (normal range, 500 to 800 mg.), cholesterol 290 mg. per 100 ml. (normal value up to 250 mg.), serum proteins 7.9 gm. per cent (albumin 4.3 gm., globulin 3.6 gm.). The serum total bilirubin was 0.4 mg. per 100 ml. with a one-minute direct serum bilirubin of 0.1 mg. A seventy-two-hour fecal urobilinogen estimation was normal. Two L.E. cell preparations were negative, and arterial oxygen saturation was normal by ear lobe oximetry. Radiologic studies of the chest, upper gastrointestinal tract, hands and pelvis were normal. An electrocardiogram was suggestive of biventricular hypertrophy and exhibited T wave and S-T segment changes compatible with myocardial ischemia. A tabulation of serial blood counts is presented in Table 1. The reticulocytes numbered 1.4 per cent. The red cell indexes were mean corpuscular volume 128 μ^3, mean corpuscular hemoglobin 33$\gamma\gamma$, mean corpuscular hemoglobin concentration 29 per cent. The peripheral blood smear revealed normal-appearing white cells. The red cells showed macrocytosis. Many normoblasts, target cells and Howell-Jolly bodies were present. The Coombs' test was negative, both direct and indirect. Sternal bone marrow aspiration revealed a hypocellular marrow with a marked decrease in the number of megakaryocytes. The red and white cell precursors were normal in morphology and ratio. There was a marked increase in fat, phagocytic cells and tissue basophils. Half-life of the patient's red cells labeled with Cr-51 and transfused into his own circulation was eighteen days. As shown in Figure 1, paper electrophoresis revealed both a rapidly migrating hemoglobin and hemoglobin A. Specimens of the patient's blood were submitted to Dr. Harvey Itano who, employing a Tiselius apparatus, demonstrated a mobility identical with a known sample of hemoglobin A-J. Solubility in 2.85 molar phosphate solution was 1.82 gm. per L., quite similar to the value of 2.00 gm. per L. reported for A-J hemoglobin. An exact quantitative separation was not achieved but the specimens were estimated to contain over 50 per cent of hemoglobin J.

TABLE 1
SERIAL STUDIES OF PERIPHERAL BLOOD REVEALING PROGRESSIVE DECREASE OF ALL CIRCULATING ELEMENTS

Date	Leukocytes (per cu. mm.)	Hemoglobin (gm. per 100 ml.)	Platelets (per cu. mm.)
July 12, 1955	5,500	12.8	41,140
August 23, 1955	6,000	12.2	.....
September 27, 1955	3,700	11.8	31,000
October 3, 1955	6,400	8.6	13,000
December 19, 1955	2,750	7.6	5,500
January 18, 1956	4,000	7.8	3,800
February 27, 1956	3,100	7.4	3,000
October 5, 1956	3,500	10.0	33,000

Four members of the patient's family were studied, including his father, mother and two younger brothers. All were asymptomatic and past medical histories were not significant. Physical examinations revealed no abnormalities except that the mother had a divergent strabismus on the left with light perception only on this side. Complete blood counts and cell morphology were normal. As shown in Figure 1, hemoglobin from all male members who were examined had patterns consistent with A-J hemoglobin identical with that of the patient. The mother's hemoglobin was normal A. The family is French-Canadian.

The patient was continued on a regimen of oral cortisone. He still complained of substernal pain on exertion and for that reason was examined by the cardiologist. Fluoroscopy revealed concentric cardiac hypertrophy with some diminution in the amplitude of ventricular contractions. Electrocardiograms taken after exercise showed a further increase in T wave and S-T segment changes which was considered indicative of myocardial ischemia. It was the consultant's opinion that the cardiac findings were not secondary to valvular deformity or septal defect but were more likely due to subendocardial fibroelastosis or an anomaly of the coronary circulation. Investigation by means of cardiac catheterization and angiocardiography was not performed because of the thrombocytopenia. During the weeks following admission new petechiae developed, and one moderately severe epistaxis necessitated nasal packing. Serial blood counts revealed a progressive decrease in all circulating blood elements. (Table 1.) Despite peripheral blood changes suggestive of hyposplenism it was decided that the increasing anemia and thrombocytopenia justified an exploratory laparotomy with removal of the spleen if one was present. The patient was prepared by transfusion of fresh, platelet-rich whole blood, handled in plastic. At the operation on January 18, an extremely small spleen located in the usual anatomic position was found. Splenectomy was performed together with biopsies of the liver, abdominal muscle and rib. The patient withstood the procedure well and no undue bleeding was encountered. The spleen was normal in shape, reddish purple in color, and fibrotic. It weighed 16 gm. and measured 6 cm. in length. Microscopically, there was marked thickening of the capsule and trabeculae with a decrease in intertrabecular area. Hemosiderosis was present throughout the organ. Marked sclerosis and intimal hyalinization of the arterioles was noted. The section of rib showed fatty marrow. Biopsy specimens of liver and muscle were normal. Postoperatively, there was no improvement in the patient's clinical status. Steroid medication was continued. When returned to full ambulation he continued to complain of chest pain. He was therefore retired from the military service and in March, 1956, was transferred to a Veterans Administration facility for further care. At the time of his departure his white blood cell count was 3,100 per cu. mm., hemoglobin 7.4 gm. per cent, and platelets 3,000 per cu. mm.

COMMENTS

To date, nine different types of hemoglobin have been described, each having distinct solubility and electrophoretic characteristics [*1–4*]. Three of these, H, I and J, exhibit mobilities more rapid than A hemoglobin during paper electrophoresis at pH 8.6. Hemoglobin J has only recently been described by Thorup et al. as an incidental finding in a young Negro girl [*1*], and it has also been reported from Liberia [*2*]. By definition, hemoglobin J has an electrophoretic mobility more rapid than A but slower than H and I. Thorup's patient exhibited the A-J trait, as did her father, brother and two sisters. Complete blood studies on the entire family were otherwise normal. The abnormal hemoglobin found in the present patient and his family seems identical with that of Thorup's patient. In view of the lack of symptoms or other hematologic abnormalities in any of the other patients with the A-J pattern, the presence of the abnormality is considered to be an incidental finding in our case.

The initial peripheral blood smears in this patient revealed the presence of normoblasts, target cells and Howell-Jolly bodies. These findings suggested that, in addition to the other abnormalities, the patient might have congenital absence or hypoplasia of the spleen. This was proved at operation. Congenital splenic aplasia occurring in adults has been described in at least ten instances by several authors [*5–7*]. There are usually other associated congenital anomalies and death frequently occurs at an early age as a result of the associated defects. Patients with minor or no associated congenital malformations have a normal life expectancy and at least one patient survived to the age of seventy-three. The peripheral blood smears in patients with congenital hypoplasia of the spleen are frequently marked by the presence of target cells, normoblasts and Howell-Jolly bodies.

Fanconi's syndrome of hypoplastic anemia with multiple congenital defects was first described in 1927. His patients and the majority of those described have been children [*13–17*], but four instances of the occurrence of this syndrome in adults have been reported. In 1949 Rohr presented case reports of two brothers [*8*]. The first died at the age of twenty-six of broncho-

pneumonia associated with severe pancytopenia, bone marrow hypoplasia and increased skin pigmentation. The patient's brother was well until the age of twenty and then pancytopenia and a hypoplastic bone marrow developed. It is of interest to note that this patient's peripheral blood smear contained normoblasts, target cells and Howell-Jolly bodies prior to splenectomy. Removal of the spleen was not followed by any significant clinical improvement. The remaining two adult patients were reported on by Cowdell, Phizackerley and Pyke in 1955 [9]. The first patient was well until the age of twenty-two when pancytopenia associated with a hypoplastic marrow developed. Numerous congenital anomalies were present and at autopsy the spleen was described as small. The brother of this patient died of acute leukemia at the age of twenty-seven and exhibited cutaneous pigmentation, bilateral deformity of the thumbs and genital hypoplasia.

In the case presented herein the patient exhibited a hypoplastic bone marrow and peripheral pancytopenia. He also had convergent strabismus, hypoplasia of the spleen and evidence of congenital heart disease. This combination of hematologic abnormality and congenital defect is strongly suggestive of Fanconi's syndrome.

SUMMARY

A third example of hemoglobin J trait is presented, with a demonstration of familial occurrence. The patient also exhibited congenital hypoplasia of the spleen. This diagnosis was entertained preoperatively on the basis of peripheral blood findings and was proved at surgery by the removal of a spleen weighing 16 gm. In addition, the patient had a combination of hematologic findings and congenital anomalies compatible with a diagnosis of Fanconi's syndrome of hypoplastic anemia.

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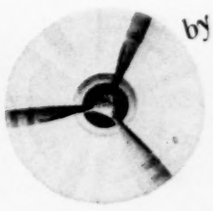
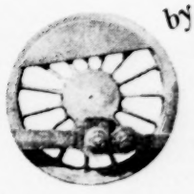
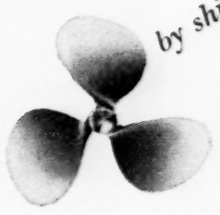
IN ANY KIND OF MOTION SICKNESS







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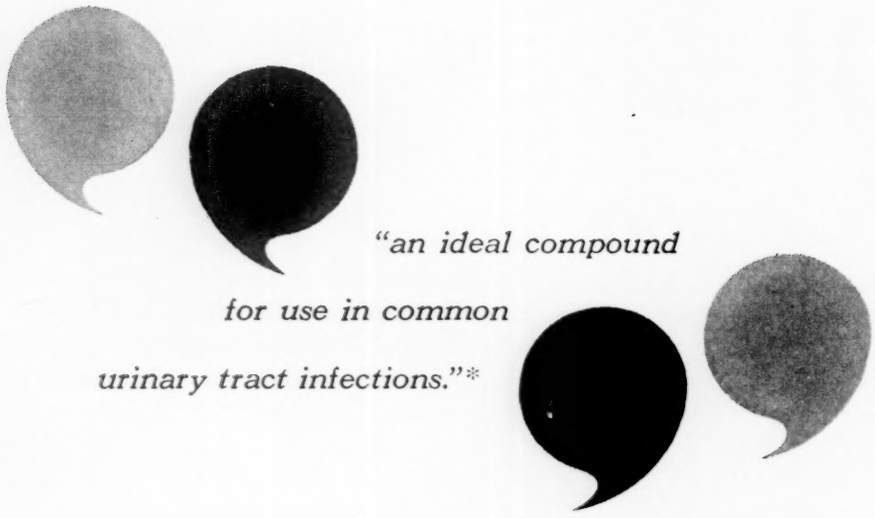

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(1) Davis, J.H. et al., Surgery, 35:434, 1954

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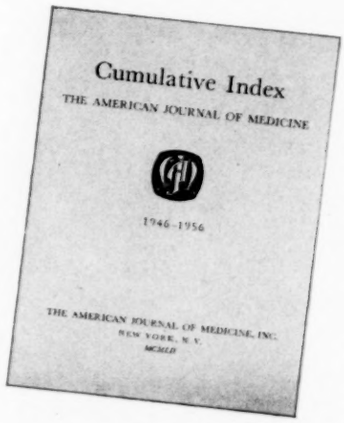

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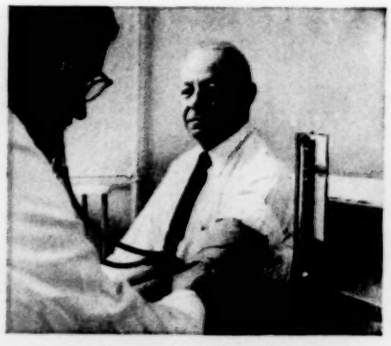

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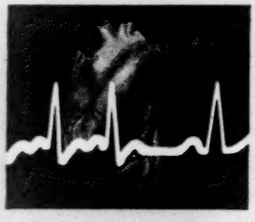

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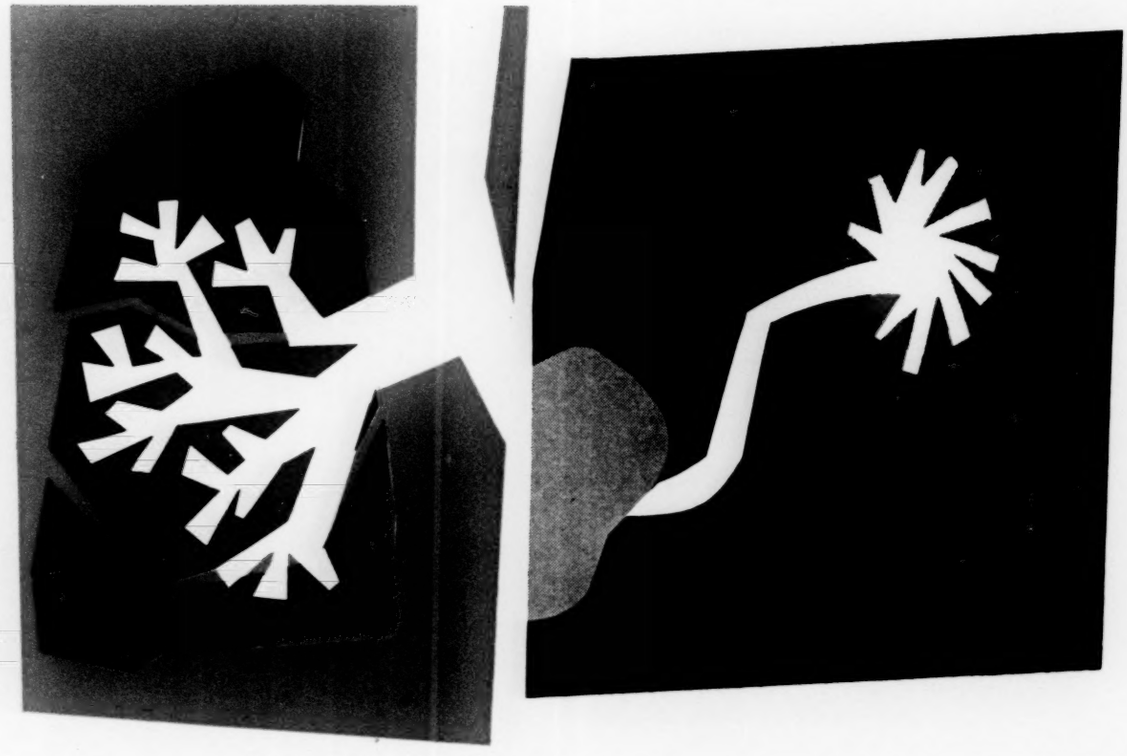

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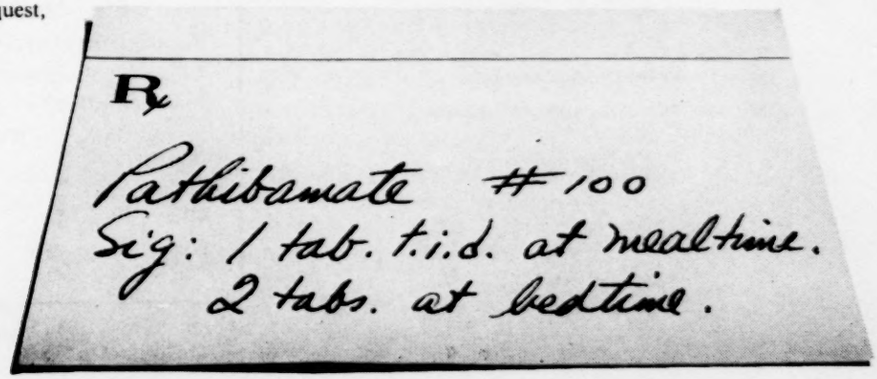

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*Lawrence, E. D.; Doktor, D., and Sall, J.: Angiology 2:405, 1951.

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1. Dickel, H. A., Wood, J. A. and Dixon, H. H.: Electromyographic studies on meprobamate and the working, anxious patient. Ann. New York Acad. Sc. **67**:780, May 9, 1957.

2. Dickel, H. A., Dixon, H. H., Wood, J. A. and Shanklin, J. G.: Electromyographic studies on patients treated with meprobamate. West. J. Surg. **64**:197, April 1956.

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DISCOVERED AND INTRODUCED BY WALLACE LABORATORIES

Miltown® TRANQUILIZER WITH MUSCLE-RELAXANT ACTION

2-methyl-2-n-propyl-1,3-propanediol dicarbamate
U. S. Patent 2,724,720

Supplied:
400 mg. scored tablets.
200 mg. sugar-coated tablets.

Literature and samples available on request.

CM-5466

WALLACE LABORATORIES, *New Brunswick, N. J.*

NEW! IMPROVED!

automatically measured-dose aerosol medications

In Asthma

and other allergic states

Both vial and Oral Adapter for Medihaler preparations are improved: The 10cc. vial for all Medihaler medications is now made of shatterproof stainless steel. The Oral Adapter is shorter, handier to use. New combination package includes Oral Adapter for patient's first prescription. No need for carrying case.

Medihaler-EPI® Riker brand epinephrine bitartrate, 7.0 mg. per cc., suspended in inert, nontoxic aerosol vehicle. Contains no alcohol. Each measured dose 0.15 mg. actual epinephrine. In 10cc. metal vial with measured-dose valve.

Indicated for quick relief of bronchospasm of any origin —asthma, bronchiectasis, emphysema.

Acts more rapidly than subcutaneous epinephrine in acute allergic reactions.

Medihaler-ISO® Riker brand isoproterenol sulfate, 2.0 mg. per cc., suspended in an inert, nontoxic aerosol vehicle. Contains no alcohol. Each measured dose 0.06 mg. actual isoproterenol. In 10cc. metal vial with measured-dose valve.

Unsurpassed for rapid relief of bronchospasm of any origin—asthma, bronchiectasis, emphysema.

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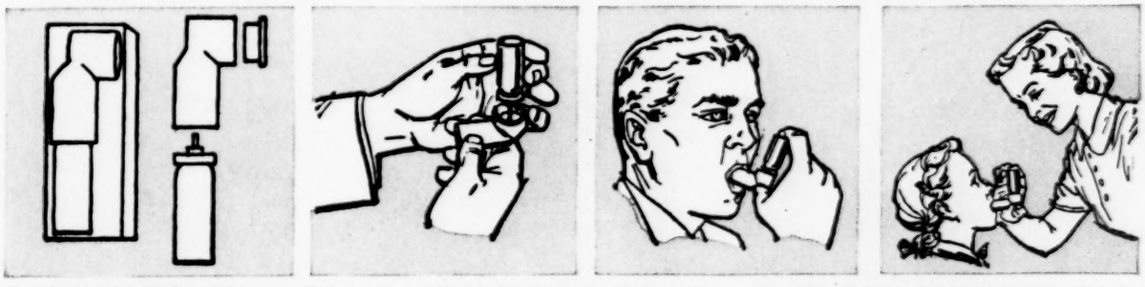

SIMPLER TO USE...RAPID, PROLONGED RELIEF...SAFE FOR CHILDREN TOO

The same automatic measured-dose principle which has made Medihaler famous. Uniform particle size. Always spillproof, leakproof; constant dosage. Now also shatterproof, and with *smaller* sterilizable, unbreakable Oral Adapter. Nothing to pour or measure. Prescribe Medihaler medication *with* Oral Adapter on first prescription. REFILLS AVAILABLE WITHOUT ORAL ADAPTER.

The Medihaler Principle

is also available in Medihaler-Phen™ (phenylephrine-hydrocortisone-neomycin) for lasting, effective relief of nasal congestion.

Riker LOS ANGELES